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INDOLOCARBAZOLE DERIVATIVES FOR SENSITIZING MULTIDRUG-RESISTANT CELLS TO ANTITUMOR AGENTS

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Abstract

Staurosporin derivatives of formula (I), wherein R1 is an acyl radical having from 2 to 30 carbon atoms, an aliphatic hydrocarbon radical having up to 29 carbon atoms that is substituted by acyclic substituents, a cycloaliphatic or cycloaliphatic-aliphatic radical each having up to 29 carbon atoms, or a heterocyclic, heterocyclic-aliphatic or heteroaliphatic radical each having up to 20 carbon atoms and up to 9 hetero atoms, R2 is hydrogen, an aliphatic, carbocyclic or carbocyclic-aliphatic radical each having up to 29 carbon atoms, a heterocyclic or heterocyclic-aliphatic radical each having up to 20 carbon atoms and up to 9 hetero atoms, or an acyl radical having up to 30 carbon atoms, and R3 is hydrogen, hydroxy, lower alkoxy or oxo, with the exception of the compound of formula (I) wherein R1 is methoxycarbonylmethyl, R2 is benzoyl and R3 is hydrogen, are described. These compounds can be used for avoiding or removing multi-drug resistance to anti-tumour agents.

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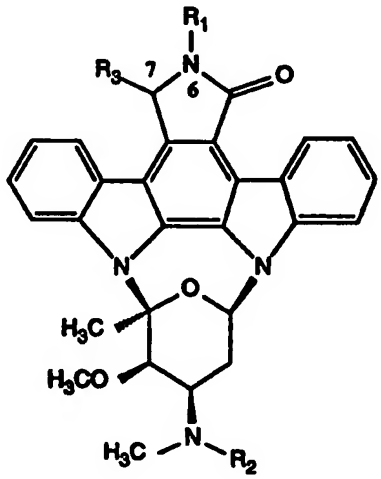
Staurosporin derivatives of formula (I), wherein R_1 is an acyl radical having from 2 to 30 carbon atoms, an aliphatic hydrocarbon radical having up to 29 carbon atoms that is substituted by acyclic substituents, a cycloaliphatic or cycloaliphatic-aliphatic radical each having up to 29 carbon atoms, or a heterocyclic, heterocyclic-aliphatic or heteroaliphatic radical each having up to 20 carbon atoms and up to 9 hetero atoms, R_2 is hydrogen, an aliphatic, carbocyclic or carbocyclic-aliphatic radical each having up to 29 carbon atoms, a heterocyclic or heterocyclic-aliphatic radical each having up to 20 carbon atoms and up to 9 hetero atoms, or an acyl radical having up to 30 carbon atoms, and R_3 is hydrogen, hydroxy, lower alkoxy or oxo, with the exception of the compound of formula (I) wherein R_1 is methoxycarbonylmethyl, R_2 is benzoyl and R_3 is hydrogen, are described. These compounds can be used for avoiding or removing multi-drug resistance to anti-tumour agents.



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INDOLOCARBAZOLE DERIVATIVES FOR SENSITIZING MULTIDRUG-RESISTANT CELLS TO ANTITUMOR AGENTS

The invention relates to staurosporin derivatives, to a process for the preparation thereof, to pharmaceutical compositions comprising such staurosporin derivatives, and to the use thereof as medicaments.

Staurosporin, which forms the basis of the derivatives according to the invention, was isolated as early as 1977 from cultures of Streptomyces staurosporeus AWAYA, TAKAHASHI and OMURA, sp. nov. AM 2282, see S. Omura *et al.*, J. Antibiot. 30, 275-281 (1977). Hitherto, only the relative, but not the absolute, configuration of staurosporin was known. The absolute configuration has been published only recently by N. Funato *et al.*, Tetrahedron Letters 35:8, 1251-1254 (1994) and corresponds to the mirror image of the structure previously used in the literature to indicate the relative configuration of staurosporin. Accordingly, the Tetrahedron Letters publication recommends verbatim "that the stereochemical notation for staurosporine which has been in common use hitherto should be revised". Although the absolute configuration was not known hitherto, it was clearly established by the term "staurosporin derivative". In this Application, the new formulae are used.

Staurosporin and most of the staurosporin derivatives known hitherto show a strong inhibitory action on protein kinase C. Protein kinase C, which is dependent upon phospholipids and calcium, occurs within the cell in several forms and participates in various fundamental processes, such as signal transmission, proliferation and differentiation and also secretion of hormones and neurotransmitters. Activation of that enzyme is brought about either by receptor-mediated hydrolysis of phospholipids of the cell membrane or by direct interaction with certain tumour-promoting active agents. The sensitivity of the cell towards receptor-mediated signal transmission can be significantly influenced by modifying the activity of protein kinase C (as the signal transmitter). Compounds that are capable of influencing the activity of protein kinase C may be used as tumour-inhibiting, anti-inflammatory, immuno-modulating and antibacterial active ingredients and may even be of interest as agents against atherosclerosis and disorders of the cardiovascular system and the central nervous system.

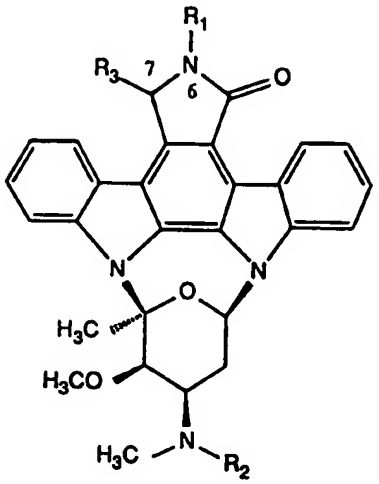
The inhibitory action on protein kinase C is weakened by a factor of from approximately 20 to over 1000 if the lactam nitrogen of staurosporin carries instead of hydrogen another substituent, that is to say, if the substituent R_1 in formula I shown hereinafter is other than

hydrogen. Especially when in formula I below the radical R_2 is, at the same time, also other than hydrogen, the inhibitory action on protein kinase C is to all practical purposes lost. When the substituent R_1 in formula I shown below is other than hydrogen, the anti-tumour activity also decreases markedly. It is presumably for that reason that only few staurosporin derivatives wherein R_1 is other than hydrogen are described in the literature, although much work has been undertaken in the field in recent years and very many derivatives wherein R_1 is hydrogen have been prepared. Thus, the compound corresponding to formula I below wherein R_1 is benzyl, R_2 is benzoyl and R_3 is hydrogen, has mostly been mentioned only as a negative control.

The appearance of resistance to classical cytostatic agents is a great problem in cancer chemotherapy. The resistance is in many cases accompanied by a reduction in the intracellular concentration of active ingredient. That reduction is often associated with the appearance of a membrane-bound 170 kilodalton glycoprotein (Pgp). That protein acts as a pump having a broad specificity and is capable of transporting frequently used anti-tumour agents, such as the Vinca alkaloids, anthracyclins, podophyllotoxins and actinomycin D, out of the cell.

Surprisingly, it has now been found that staurosporin derivatives of formula I shown hereinbelow are capable of fully re-sensitising multidrug-resistant cells to the action of anti-tumour agents, such as cytostatics, as can be demonstrated *inter alia* by the example of resistant human KB-8511 cells. This is achieved even though, as mentioned above, all derivatives show a greatly weakened inhibitory action or no inhibitory action at all on protein kinase C and the anti-tumour activity is also markedly reduced. Also surprising is the high degree of sensitisation. In that respect, the staurosporin derivatives of formula I are roughly equivalent to the analogous derivatives wherein R_1 is hydrogen. Compared with a combination of a conventional cytostatic agent and a staurosporin derivative having pronounced anti-tumour activity and inhibitory action on protein kinase C, a combination of a conventional cytostatic agent with a staurosporin derivative of formula I shown hereinbelow has the advantage that the side-effects associated with the protein kinase C inhibitory action do not occur or occur only in a very much weaker form. The administration of protein kinase C inhibiting staurosporin derivatives results, for example in dogs, in nausea to the point of vomiting. The latter is understandably disadvantageous, especially for an orally administered anti-tumour agent, since active ingredient may also be vomited, with the result that the dose of active ingredient effectively taken may be different from the intended and administered dose.

The invention relates to staurosporin derivatives of formula I



(I)

wherein

R_1 is an acyl radical having from 2 to 30 carbon atoms, an aliphatic hydrocarbon radical having up to 29 carbon atoms that is substituted by acyclic substituents, a cycloaliphatic or cycloaliphatic-aliphatic radical each having up to 29 carbon atoms, or a heterocyclic, heterocyclic-aliphatic or heteroaliphatic radical each having up to 20 carbon atoms and up to 9 hetero atoms,

R_2 is hydrogen, an aliphatic, carbocyclic or carbocyclic-aliphatic radical each having up to 29 carbon atoms, a heterocyclic or heterocyclic-aliphatic radical each having up to 20 carbon atoms and up to 9 hetero atoms, or an acyl radical having up to 30 carbon atoms, and

R_3 is hydrogen, hydroxy, lower alkoxy or oxo,

and salts of such compounds of formula I having at least one salt-forming group, with the exception of the compound of formula I wherein R_1 is methoxycarbonylmethyl, R_2 is benzoyl and R_3 is hydrogen.

An acyl radical R_1 having from 2 to 30 carbon atoms is derived from an optionally functionally modified carboxylic acid, an organic sulfonic acid or a free or esterified phosphoric acid, such as pyro- or ortho-phosphoric acid.

An acyl derived from an optionally functionally modified carboxylic acid, which is

designated Ac^1, is especially one of the partial formula Z-C(=W)- wherein W is oxygen, sulfur or imino, and Z is hydrocarbyl R^o having up to 29 carbon atoms, hydrocarbyloxy R^o-O- or an amino group, especially one of the formula $R_4(R_5)N$-.

The hydrocarbyl (hydrocarbon radical) R^o is an acyclic (aliphatic), carbocyclic or carbocyclic-acyclic hydrocarbon radical having up to 29 carbon atoms, especially up to 18, and preferably up to 12, carbon atoms and is saturated or unsaturated and unsubstituted or substituted. It may also contain in place of one, two or more carbon atoms identical or different hetero atoms, such as especially oxygen, sulfur and nitrogen, in the acyclic and/or cyclic moiety; in the latter case, it is referred to as a heterocyclic radical (heterocyclyl radical) or a heterocyclic-acyclic radical.

Unsaturated radicals are those which contain one or more, especially conjugated and/or isolated, multiple bonds (double and/or triple bonds). The term "cyclic radicals" also includes aromatic radicals, for example those wherein at least one 6-membered carbocyclic or one 5- to 8-membered heterocyclic ring contains the maximum number of non-cumulated double bonds. Carbocyclic radicals wherein at least one ring is in the form of a 6-membered aromatic ring (i.e. a benzene ring) are referred to as aryl radicals.

Unless stated otherwise, in this disclosure, organic radicals referred to as "lower" contain not more than 7, preferably not more than 4, carbon atoms.

An acyclic unsubstituted hydrocarbon radical is especially a straight-chain or branched lower alkyl, lower alkenyl, lower alkadienyl or lower alkynyl radical. Lower alkyl is, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl, or also n-pentyl, isopentyl, n-hexyl, isohexyl or n-heptyl; lower alkenyl is, for example, allyl, propenyl, isopropenyl, 2- or 3-methallyl or 2- or 3-butenyl; lower alkadienyl is, for example, 1-penta-2,4-dienyl; lower alkynyl is, for example, propargyl or 2-butynyl. In corresponding unsaturated radicals, the double bond is located especially in a position higher than the α-position with respect to the free valency.

A carbocyclic hydrocarbon radical is especially a mono-, bi- or poly-cyclic cycloalkyl, cycloalkenyl or cycloalkadienyl radical, or a corresponding aryl radical. Preference is given to radicals having a maximum of 14, especially 12, ring carbon atoms and 3- to 8-, preferably 5- to 7- and especially 6-membered rings, it also being possible for them to carry one or more, for example two, acyclic radicals, for example those mentioned above,

and especially the lower alkyl radicals, or further carbocyclic radicals. Carbocyclic-acyclic radicals are those in which an acyclic radical, especially one having a maximum of 7, preferably a maximum of 4, carbon atoms, such as especially methyl, ethyl and vinyl, carries one or more carbocyclic, if desired aromatic, radicals as defined above. There may be mentioned, in particular, cycloalkyl-lower alkyl and aryl-lower alkyl radicals, and the analogues thereof that are unsaturated in the ring and/or chain and that carry the ring at the terminal carbon atom of the chain.

Cycloalkyl has especially from 3 up to and including 10 carbon atoms and is, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl, and also bicyclo[2,2,2]octyl, 2-bicyclo[2,2,1]heptyl and adamantyl, each of which may also be substituted by 1, 2 or more, for example lower, alkyl radicals, especially methyl radicals; cycloalkenyl is, for example, one of the monocyclic cycloalkyl radicals already mentioned that has a double bond in the 1-, 2- or 3-position. Cycloalkyl-lower alkyl or -lower alkenyl is, for example, methyl, 1- or 2-ethyl, 1- or 2-vinyl, 1-, 2- or 3-propyl or allyl substituted by one of the above-mentioned cycloalkyl radicals, those substituted at the end of the linear chain being preferred.

An aryl radical is especially phenyl, but also naphthyl, such as 1- or 2-naphthyl, biphenyl-yl, such as especially 4-biphenylyl, or also anthryl, fluorenyl or azulenyl, or an aromatic analogue thereof having one or more saturated rings. Preferred aryl-lower alkyl and aryl-lower alkenyl radicals are, for example, phenyl-lower alkyl or phenyl-lower alkenyl with a terminal phenyl radical, for example benzyl, phenethyl, 1-, 2- or 3-phenylpropyl, diphenylmethyl (benzhydryl), trityl and cinnamyl, and also 1- or 2-naphthylmethyl. Of the aryl radicals that carry acyclic radicals, such as lower alkyl, there are to be mentioned, in particular, o-, m- and p-tolyl and xylyl radicals having methyl radicals situated in different positions.

Heterocyclic radicals, including heterocyclic-acyclic radicals, are especially monocyclic, but also bi- or poly-cyclic, aza-, thia-, oxa-, thiaza-, oxaza-, diaza-, triaza or tetraza-cyclic radicals of aromatic character, and corresponding partially saturated or, especially, completely saturated heterocyclic radicals of that kind, it being possible for such radicals, where appropriate, for example like the above-mentioned carbocyclic or aryl radicals, to carry further acyclic, carbocyclic or heterocyclic radicals and/or to be mono-, di- or poly-substituted by functional groups. The acyclic moiety in heterocyclic-acyclic radicals is, for example, as defined in the case of the corresponding carbocyclic-acyclic radicals.

Such radicals are, especially, unsubstituted or substituted monocyclic radicals having one nitrogen, oxygen or sulfur atom, such as 2-aziridinyl, and especially aromatic radicals of that kind, such as pyrryl, for example 2-pyrryl or 3-pyrryl, pyridyl, for example 2-, 3- or 4-pyridyl, or also thienyl, for example 2- or 3-thienyl, or furyl, for example 2-furyl; analogous bicyclic radicals having one nitrogen, oxygen or sulfur atom are, for example, indolyl, such as 2- or 3-indolyl, quinolyl, such as 2- or 4-quinolyl, isoquinolyl, such as 3- or 5-isoquinolyl, benzofuranyl, such as 2-benzofuranyl, chromenyl, such as 3-chromenyl, or benzothienyl, such as 2- or 3-benzothienyl; preferred monocyclic and bicyclic radicals having a plurality of hetero atoms are, for example, imidazolyl, such as 2-imidazolyl, pyrimidinyl, such as 2- or 4-pyrimidinyl, oxazolyl, such as 2-oxazolyl, isoxazolyl, such as 3-isoxazolyl, or thiazolyl, such as 2-thiazolyl, and benzimidazolyl, such as 2-benzimidazolyl, benzoxazolyl, such as 2-benzoxazolyl, or quinazolyl, such as 2-quinazolinyl. Corresponding partially saturated or, especially, completely saturated analogous radicals also come into consideration, such as 2-tetrahydrofuryl, 4-tetrahydrofuryl, 2- or 3-pyrrolidyl, 2-, 3- or 4-piperidyl, and also 2- or 3-morpholinyl, 2- or 3-thiomorpholinyl, 2-piperazinyl and N,N'-bis-lower alkyl-2-piperazinyl radicals. These radicals may also carry one or more acyclic, carbocyclic or heterocyclic radicals, especially those mentioned above. Heterocyclic-acyclic radicals are derived especially from acyclic radicals having a maximum of 7, preferably a maximum of 4, carbon atoms, for example from those mentioned above, and may carry one, two or more heterocyclic radicals, for example those mentioned above, it also being possible for the ring to be bonded to the chain by one of its nitrogen atoms.

As has already been mentioned, a hydrocarbyl (including a heterocyclyl) may be substituted by one, two or more substituents (functional groups) of the same kind or of different kinds; the following substituents come into consideration especially: free, etherified and esterified hydroxy groups; mercapto, lower alkylthio and unsubstituted or substituted phenylthio groups; halogen atoms, such as chlorine and fluorine, but also bromine and iodine; oxo groups that are in the form of formyl (i.e. aldehydo) and keto groups and in the form of corresponding acetals and ketals, respectively; azido and nitro groups; primary, secondary and, preferably, tertiary amino groups, primary and secondary amino groups that are protected by conventional protecting groups, acylamino groups and diacylamino groups, and free or functionally modified sulfo groups, such as sulfamoyl groups or sulfo groups in salt form. None of these functional groups should be located at the carbon atom from which the free valency extends and they are all preferably separated therefrom by 2 or even more carbon atoms. The hydrocarbyl radical may also carry free and functionally

modified carboxy groups, such as carboxy groups in salt form or esterified carboxy groups, or carbamoyl, ureido or guanidino groups optionally carrying one or two hydrocarbon radicals, and cyano groups.

An etherified hydroxy group present as a substituent in the hydrocarbyl is, for example, a lower alkoxy group, such as a methoxy, ethoxy, propoxy, isopropoxy, butoxy or tert-butoxy group, which may also be substituted. For example, such a lower alkoxy group may be substituted, for example mono-, di- or poly-substituted, by halogen atoms, especially in the 2-position, such as in the 2,2,2-trichloroethoxy, 2-chloroethoxy and 2-iodoethoxy radical, or substituted, preferably mono-substituted, by hydroxy or by lower alkoxy radicals, especially in the 2-position, such as in the 2-methoxyethoxy radical. An especially preferred form of the etherified hydroxy groups exists in oxa-alkyl radicals, in which one or more of the carbon atoms in a preferably linear alkyl have been replaced by oxygen atoms that are preferably separated from one another by a plurality of (especially 2) carbon atoms, so that they form an optionally repeatedly recurring $(-O-CH_2-CH_2)_n-$ group wherein n = 1 to 14. Such etherified hydroxy groups are also unsubstituted or substituted phenoxy radicals and phenyl-lower alkoxy radicals, such as especially benzyloxy, benzhydryloxy and triphenylmethoxy (trityloxy), and heterocyclyloxy radicals, such as especially 2-tetrahydropyranyloxy. The groupings methylenedioxy and ethylenedioxy may be regarded as special etherified hydroxy groups; the former as a rule bridges 2 adjacent carbon atoms, especially in aryl radicals, and the latter is bonded to one and the same carbon atom and may be regarded as a protecting group for oxo.

The expression "etherified hydroxy groups" is also to be understood in this context as including silylated hydroxy groups, as occur, for example, in tri-lower alkylsilyloxy, such as trimethylsilyloxy and dimethyl-tert-butylsilyloxy, or phenyl-di-lower alkylsilyloxy or lower alkyl-diphenylsilyloxy.

An esterified hydroxy group present as a substituent in the hydrocarbyl is, for example, lower alkanoyloxy.

An esterified carboxy group present as a substituent in the hydrocarbyl is one in which the hydrogen atom has been replaced by one of the hydrocarbon radicals characterised above, preferably a lower alkyl or phenyl-lower alkyl radical; there may be mentioned as examples of an esterified carboxy group lower alkoxycarbonyl or phenyl-lower alkoxycarbonyl that is unsubstituted or substituted in the phenyl moiety, especially the methoxy-,

ethoxy-, tert-butoxy- or benzyloxy-carbonyl group, and also a lactonised carboxy group.

A primary amino group $-NH_2$ as a substituent of the hydrocarbyl may also be in protected form. A secondary amino group carries, in place of one of the two hydrogen atoms, a hydrocarbyl radical, preferably an unsubstituted hydrocarbyl radical, such as one of those mentioned above, especially lower alkyl, and may also be in protected form.

A tertiary amino group occurring as a substituent in the hydrocarbyl carries 2 different or, preferably, identical hydrocarbyl radicals (including the heterocyclic radicals), such as the unsubstituted hydrocarbyl radicals characterised above, especially lower alkyl.

A preferred amino group is one of the formula $R_4(R_5)N-$, wherein R_4 and R_5 are each independently of the other hydrogen, unsubstituted acyclic C_1-C_7hydrocarbyl (such as, especially, a C_1-C_4alkyl or C_2-C_4alkenyl) or monocyclic aryl, aralkyl or aralkenyl that has a maximum of 10 carbon atoms and that is unsubstituted or substituted by C_1-C_4alkyl, C_1-C_4alkoxy, halogen and/or by nitro, it being possible for the carbon-containing radicals to be bonded to one another by a carbon-carbon bond or by an oxygen atom, by a sulfur atom, or by a nitrogen atom that is unsubstituted or substituted by hydrocarbyl. In such a case, they form together with the nitrogen atom of the amino group a nitrogen-containing heterocyclic ring. The following may be mentioned as examples of especially preferred free amino groups: di-lower alkylamino, such as dimethylamino, diethylamino, pyrrolidino, piperidino, morpholino, thiomorpholino and piperazino or 4-methylpiperazino, or diphenylamino and dibenzylamino each unsubstituted or substituted, especially in the phenyl moiety, for example by lower alkyl, lower alkoxy, halogen and/or by nitro; and, of the protected amino groups, especially lower alkoxycarbonylamino, such as tert-butoxycarbonylamino, phenyl-lower alkoxycarbonylamino, such as 4-methoxybenzyloxycarbonylamino, and 9-fluorenylmethoxycarbonylamino.

Unless stated otherwise, hereinbefore and hereinafter aromatic carbocyclic and heterocyclic hydrocarbyl radicals may be mono- or poly-substituted, such as di- or trisubstituted, especially by C_1-C_4alkyl, C_1-C_4alkoxy, halogen, nitro, trifluoromethyl, also carboxy, C_1-C_4alkoxycarbonyl, methylenedioxy and/or by cyano. Substituents mentioned specifically hereinbefore and hereinafter are to be regarded as preferences.

Preferred compounds of formula I according to the invention are, for example, those wherein hydrocarbyl R^o has the following preferred meanings of an acyclic hydrocarbyl: a

C_1-C_{20}alkyl, a C_2-C_{20}hydroxyalkyl the hydroxy group of which is located in any position apart from the 1-position, preferably in the 2-position, a cyano-[C_1-C_{20}]alkyl the cyano group of which is preferably located in the 1- or ω-position, or a carboxy-[C_1-C_{20}]alkyl the carboxy group of which is preferably located in the 1- or ω-position and may, where appropriate, also be in salt form or in the form of a C_1-C_4alkyl ester (C_1-C_4alkoxy-carbonyl) or a benzyl ester (benzyloxycarbonyl), and a C_3-C_{20}alkenyl the free valency of which is not located at the same carbon atom as is the double bond, all of the mentioned radicals, with the exception of those with the C_3-C_5alkyl basic structure, having a linear (unbranched) alkyl chain; also a linear (mono-, di- to hexa)-oxaalkyl having from 4 to 20 chain members, wherein one or more of the carbon atoms, from C-3 onward, of a linear C_4-C_{20}alkyl has been replaced by oxygen atoms that are separated from one another by at least 2 carbon atoms and are preferably located in positions 3, 6, 9, 12, 15 and 18.

Preferred compounds of formula I according to the invention are also those wherein hydrocarbyl R^o has the following preferred meanings of a carbocyclic or heterocyclic, or also carbocyclic-acyclic or heterocyclic-acyclic, hydrocarbyl: a bicyclic or, preferably, monocyclic aryl, especially phenyl, or also naphthyl, that may carry one or more of the following substituents: halogen atoms, especially fluorine, chlorine and bromine, C_1-C_4-alkyl radicals, especially methyl, C_1-C_4alkoxy groups, especially methoxy, methylene-dioxy, nitro groups and/or carboxy groups that may be free, in salt form or in the form of C_1-C_4alkyl esters, especially methoxycarbonyl or ethoxycarbonyl. Preferably, the aryl radicals carry not more than 2 substituents, especially those of the same kind, or carry only a single substituent; most especially, they are unsubstituted. A preferred heterocyclic hydrocarbyl (hydrocyclyl) is, for example, one that is analogous to the aryl radicals given prominence above and that contains, instead of one or two carbon atoms, in each case a hetero atom, especially nitrogen, such as a pyridyl or quinolyl, or quinazolyl, respectively, wherein the free valency is located at a carbon atom and accordingly can also be substituted. Preferred carbocyclic-acyclic and heterocyclic-acyclic hydrocarbyl radicals are those wherein two or three, but preferably only one, of the above-defined cyclic radicals, preferably the unsubstituted cyclic radicals, is carried by a C_1-C_3alkyl, all of them preferably being located at one carbon atom, preferably the terminal carbon atom; unsubstituted benzyl is most preferred.

Especially preferred compounds of formula I are those wherein R^o is C_1-C_7alkyl, especially C_1-C_4alkyl, hydroxy-C_2-C_{18}alkyl, especially hydroxy-C_2-C_{14}alkyl, cyano-C_1-C_7alkyl, especially cyano-C_1-C_4alkyl, carboxy-C_1-C_7alkyl, especially carboxy-C_1-C_4alkyl, C_1-C_7-

alkoxy-carbonyl-C_1-C_7alkyl, especially C_1-C_4alkoxy-carbonyl-C_1-C_4alkyl, benzyloxy-carbonyl-C_1-C_7alkyl, especially benzyloxycarbonyl-C_1-C_4alkyl, C_3-C_7alkenyl, phenyl, naphthyl, pyridyl, quinolyl, or quinazolyl, or phenyl-C_1-C_7alkyl, especially phenyl-C_1-C_3-alkyl, it also being possible for the respective aromatic radicals to be substituted by C_1-C_7alkyl, especially C_1-C_4alkyl, C_1-C_7alkoxy, especially C_1-C_4alkoxy, halogen, nitro, trifluoromethyl, also carboxy, C_1-C_4alkoxy-carbonyl, methylenedioxy and/or by cyano, the hydroxy group in a correspondingly substituted alkyl radical being located especially in the 2-position, and the cyano, carboxy, alkoxy-carbonyl, benzyloxy-carbonyl or phenyl group in a correspondingly substituted alkyl radical being located in the 1- or ω-position.

Especially preferred compounds of formula I are those wherein R^o is C_1-C_4alkyl, such as methyl or ethyl, hydroxy-C_2-C_{14}alkyl, such as 2-hydroxy-propyl, -hexyl, -decyl or -tetradecyl, cyano-C_1-C_4alkyl, such as 2-cyanoethyl, carboxy-C_1-C_4alkyl, such as carboxymethyl, C_1-C_4alkoxycarbonyl-C_1-C_4alkyl, such as methoxycarbonyl-methyl or -ethyl, C_3-C_7alkenyl, such as allyl, or phenyl, the hydroxy group in a correspondingly substituted alkyl preferably being located in the 2-position and the cyano, carboxy or alkoxycarbonyl group being located especially in the 1- or ω-position.

An acyl derived from an organic sulfonic acid, which is designated Ac^2, is especially one of the partial formula R^o-SO_2- wherein R^o is a hydrocarbyl having the general meanings given above and the meanings given prominence above, the latter meanings generally representing also in this case the preferred selection.

An acyl derived from a free or esterified phosphoric acid, which is designated Ac^3, is especially one of the partial formula $R_4O(R_5O)P(=O)$-, wherein R_4 and R_5 each independently have the general meanings given above and the meanings given prominence above. R_4 and R_5 preferably have the same meaning.

Preferred acyl radicals Ac^1 are acyl radicals of a carboxylic acid that are characterised by the partial formula R^o-CO- wherein R^o has one of the above-mentioned general and preferred meanings of the hydrocarbyl radical R^o, and that are accordingly derived from an unsubstituted or substituted acyclic, carbocyclic, carbocyclic-acyclic, heterocyclic or heterocyclic-acyclic monocarboxylic acid. A preferred hydrocarbyl in such an acyl is, for example, a C_1-C_{19}alkyl, especially a C_1-C_7- or C_1-C_4-alkyl, especially one that, in the case of more than 5 carbon atoms, has a linear chain and that may also carry the following substituents: a carboxy group that may also be in salt form or in the form of a cyano group or

a C_1-C_4alkyl ester (C_1-C_4alkoxycarbonyl group) and that is preferably located in the ω-position, an amino group of the above-defined formula $R_4(R_5)N$-, preferably one in which R_4 and R_5 are each hydrogen and that is then preferably located in the 1-position, or one or more halogen atoms, especially fluorine or chlorine, which are preferably located vicinal to the carbonyl group. Another preferred acyl is a bicyclic or, especially, monocyclic aroyl, especially benzoyl, that may also carry one or more of the following substituents: halogen atoms, especially chlorine or fluorine, nitro groups, C_1-C_4alkyl radicals, especially methyl, hydroxy groups and etherified hydroxy groups, especially C_1-C_4alkoxy, such as methoxy, phenoxy and methylenedioxy, and carboxy groups that may also be in salt form or in the form of a cyano group or a C_1-C_4alkyl ester (C_1-C_4alkoxycarbonyl). Preferably, the aroyl radicals carry not more than 2, and especially only one, such substituent. Also preferred are analogous heteroaroyl radicals, especially those derived from pyridine, pyrrole, furan, thiophene and imidazole and from analogues thereof having a fused benzo ring (such as quinoline, isoquinoline, benzofuran and benzimidazole) and that are also unsubstituted or substituted as indicated above. Preferred acyl radicals of that kind are also derived from monocyclic aryl-alkenyl, for example corresponding aryl-C_2-C_5alkenyl, such as benzyl and styryl (i.e. phenacetyl and cinnamoyl) and can also be substituted in the manner given above. There may be mentioned by way of example acyl radicals R_1 that are derived from the following carboxylic acids: aliphatic monocarboxylic acids having a maximum of 20 carbon atoms, such as lower alkanecarboxylic acids, for example propionic, butyric, isobutyric, valeric, isovaleric, caproic, trimethylacetic, oenanthic and diethylacetic acid and, especially, acetic acid, and lauric, myristic, palmitic and stearic acid, and oleic acid, elaidic acid, linoleic acid and linolenic acid, but also corresponding halogenated lower alkanecarboxylic acids, such as chloroacetic acid, trifluoro- or trichloro-acetic acid, bromoacetic or α-bromoisovaleric acid; carbocyclic or carbocyclic-acyclic monocarboxylic acids, for example cyclopropane-, cyclopentane- and cyclohexane-carboxylic acid or cyclopentane- or cyclohexane-acetic acid or -propionic acid, respectively; aromatic carbocyclic carboxylic acids, for example benzoic acid that may be mono- or poly-substituted as indicated above; aryl- or aryloxy-lower alkanecarboxylic acids and analogues thereof that are unsaturated in the chain, for example phenylacetic or phenoxyacetic acids that are unsubstituted or substituted as indicated above for benzoic acid, phenylpropionic acids and cinnamic acids; and heterocyclic acids, for example furan-2-carboxylic acid, 5-tert-butylfuran-2-carboxylic acid, thiophene-2-carboxylic acid, nicotinic or isonicotinic acid, 4-pyridinepropionic acid, and pyrrole-2- or -3-carboxylic acids that are unsubstituted or substituted by lower alkyl radicals; also corresponding α-amino acids, especially naturally occurring α-amino acids of the L-series, for example

glycine, phenylglycine, alanine, phenylalanine, proline, leucine, serine, valine, tyrosine, arginine, histidine and asparagine, preferably in an N-protected form, i.e. in a form in which the amino group is substituted by conventional, for example one of the above-mentioned, amino-protecting groups; and also dicarboxylic acids, such as oxalic acid, malonic acid, mono- or di-lower alkylmalonic acids, succinic acid, glutaric acid, adipic acid, erucic acid, maleic acid, or a phthalic, quinolinic, isoquinolinic or phenylsuccinic acid that is unsubstituted or substituted by halogen, such as fluorine, chlorine or bromine, and/or by lower alkyl, hydroxy, lower alkoxy and by nitro, and also glutamic acids and aspartic acid, the latter two acids preferably having protected amino groups. As mentioned, the second carboxy group may not only be free but may also be functionally modified, for example in the form of a C_1-C_4alkyl ester or in the form of a salt, preferably in the form of a physiologically tolerable salt, with a salt-forming basic component. There come into consideration especially metal or ammonium salts, such as alkali metal and alkaline earth metal salts, for example sodium, potassium, magnesium or calcium salts, or ammonium salts with ammonia or suitable organic amines.

Another preferred acyl Ac^1 is derived from monoesters of carbonic acid and is characterised by the partial formula R^o-O-CO-. Among especially preferred hydrocarbyl radicals R^o in these derivatives there are to be mentioned, for example, the following: acyclic hydrocarbyl, especially a C_1-C_{20}alkyl, preferably a linear C_1-C_{20}alkyl, that may be substituted by a carboxy group, preferably in a functionally modified form, such as a salt, cyano or a C_1-C_4alkyl ester, that is preferably located in the ω-position, or an analogous linear (mono- to hexa-)oxaalkyl having from 4 to 20 chain members, especially one characterised above as being especially preferred. Also preferred within this definition of R^o are unsubstituted or substituted phenyl and benzyl radicals, for example those mentioned above as being preferred.

Yet another preferred acyl Ac^1 is derived from amides of carbonic acid (or also thiocarbonic acid) and is characterised by the formula $R_4(R_5)N$-C(=W)- wherein R_4 and R_5 are as defined above and W is sulfur or especially oxygen.

The acyl radical Ac^2 is derived from an acyclic, carbocyclic or heterocyclic, or also a carbocyclic-acyclic or heterocyclic-acyclic sulfonic acid and corresponds to the mentioned partial formula R^o-SO_2- wherein R^o is hydrocarbyl having the above-mentioned general and, especially, the preferred meanings. Of the compounds according to the invention that carry the radical Ac^2 special prominence is to be given to those wherein R^o is a

C_1-C_7alkyl, or, especially, a bicyclic or especially a monocyclic aryl, such as especially phenyl, that may be substituted in a manner analogous to that described above for the aroyl radicals given prominence. Prominence is also to be given to bicyclic and monocyclic aromatic heterocyclyl radicals of analogous structure, in which one or two of the carbon atoms have been replaced by hetero atoms, such as pyrimidyl, for example 2- or 4-pyrimidyl, quinolyl or isoquinolyl. The heterocyclyl radicals also may carry substituents, especially those given prominence for aroyl (in that case, for example, a hydroxy derivative is, by virtue of tautomeric shifting of the double bond, the same as a dihydro-oxo derivative).

The acyl radical Ac^3 derived from a phosphoric acid is, for example, an acyl radical that is derived from pyrophosphoric acid or, especially, from orthophosphoric acid and that may also be in a functionally modified form, for example in the form of a salt, a hydrocarbyl ester or an amide. Of the compounds of formula I according to the invention wherein R_1 is Ac^3 prominence is to be given especially to those wherein Ac^3 corresponds to the partial formula $R_4O(R_5O)P(=O)$- wherein R_4 and R_5 have the general meanings given above and the meanings given special prominence above and are preferably identical and are hydrogen or an unsubstituted C_1-C_7alkyl, especially a linear C_1-C_7alkyl, such as especially methyl or ethyl, or alternatively phenyl that is unsubstituted or substituted, especially by C_1-C_4alkyl, C_1-C_4alkoxy, halogens and/or by nitro.

Especially preferred are those compounds of formula I wherein R_1 is an acyl of the partial formula Z-C(=W)- wherein W is oxygen, also sulfur, and Z is C_1-C_7alkyl that may also be substituted by halogen, carboxy or by C_1-C_4alkoxy-carbonyl.

Especially preferred are those compounds of formula I wherein R_1 is an acyl of the partial formula Z-C(=W)- wherein W is oxygen or also sulfur, and Z is phenyl, or also pyridyl, furyl, thienyl, imidazolyl, quinolyl, isoquinolyl, benzofuranyl or benzimidazolyl, each of which, including phenyl, is unsubstituted or substituted by C_1-C_4alkyl, C_1-C_4alkoxy, halogen, nitro, trifluoromethyl, carboxy, C_1-C_4alkoxy-carbonyl, methylenedioxy and/or by cyano.

Especially preferred are those compounds of formula I wherein R_1 is an acyl of the partial formula R^o-CO- wherein R^o is C_1-C_7alkyl, especially C_1-C_4alkyl, such as methyl or tert-butyl, that may also be substituted by halogen, such as fluorine or chlorine, carboxy or by C_1-C_4alkoxy-carbonyl, such as methoxycarbonyl, such as trifluoro- or trichloro-methyl,

2-carboxy- or 2-methoxycarbonyl-ethyl.

Especially preferred are those compounds of formula I wherein R_1 is an acyl of the partial formula R°-CO- wherein R° is phenyl that is unsubstituted or that may also be substituted by C_1-C_4alkyl, C_1-C_4alkoxy, halogen, such as fluorine or chlorine, nitro, trifluoromethyl, carboxy or by C_1-C_4alkoxy-carbonyl.

Especially preferred are those compounds of formula I wherein R_1 is an acyl of the partial formula R°-SO_2- wherein R° is C_1-C_7alkyl, especially C_1-C_4alkyl.

Especially preferred are those compounds of formula I wherein R_1 is an acyl of the partial formula R°-SO_2- wherein R° is phenyl, or also pyridyl, furyl, thienyl, imidazolyl, quinolyl, isoquinolyl, benzofuranyl or benzimidazolyl, each of which is unsubstituted or substituted by C_1-C_4alkyl, C_1-C_4alkoxy, halogen, nitro, trifluoromethyl, carboxy, C_1-C_4alkoxy-carbonyl, methylenedioxy and/or by cyano.

Especially preferred are those compounds of formula I wherein R_1 is an acyl of the partial formula R°-SO_2- wherein R° is phenyl or C_1-C_4alkyl- or halo-substituted phenyl or isoquinolyl, such as 5-isoquinolyl.

Especially preferred are those compounds of formula I wherein R_1 is an acyl of the partial formula R°-O-CO- wherein R° is C_1-C_7alkyl, especially C_1-C_4alkyl.

Especially preferred are those compounds of formula I wherein R_1 is an acyl of the partial formula R°-O-CO- wherein R° is phenyl, or also pyridyl, furyl, thienyl, imidazolyl, quinolyl, isoquinolyl, benzofuranyl or benzimidazolyl, each of which is unsubstituted or substituted by C_1-C_4alkyl, C_1-C_4alkoxy, halogen, nitro, trifluoromethyl, carboxy, C_1-C_4-alkoxy-carbonyl, methylenedioxy and/or by cyano.

Especially preferred is the compound of formula I wherein R_1 is an acyl of the partial formula R°-O-CO- wherein R° is unsubstituted phenyl.

Especially preferred are those compounds of formula I wherein R_1 is an acyl of the partial formula $R_4(R_5)N$-C(=W)- wherein W is sulfur or, especially, oxygen, R_4 is hydrogen and R_5 is C_1-C_7alkyl, especially C_1-C_4alkyl, C_3-C_7alkenyl or phenyl, or also pyridyl, furyl, thienyl, imidazolyl, quinolyl, isoquinolyl, benzofuranyl or benzimidazolyl, each of which

is unsubstituted or substituted by C_1-C_4alkyl, C_1-C_4alkoxy, halogen, nitro, trifluoromethyl, carboxy, C_1-C_4alkoxy-carbonyl, methylenedioxy and/or by cyano.

Especially preferred are those compounds of formula I wherein R_1 is derived from an α-amino acid, especially a naturally occurring α-amino acid of the L-series.

Especially preferred are those compounds of formula I wherein R_1 is derived from an α-amino acid selected from glycine, phenylglycine, alanine, phenylalanine, proline, leucine, serine, valine, tyrosine, arginine, histidine and asparagine.

Especially preferred are those compounds of formula I wherein R_1 is derived from an α-amino acid selected from glycine, alanine, phenylalanine, serine, arginine and histidine.

An aliphatic hydrocarbon radical R_1 having up to 29 carbon atoms that is substituted by acyclic substituents has preferably a maximum of 18, especially a maximum of 12 and, as a rule, not more than 7, carbon atoms, can be saturated or unsaturated and is especially a linear or branched lower alkyl, lower alkenyl, lower alkadienyl or lower alkynyl radical that is substituted by acyclic substituents. Lower alkyl is, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl, or also n-pentyl, isopentyl, n-hexyl, isohexyl or n-heptyl; lower alkenyl is, for example, allyl, propenyl, isopropenyl, 2- or 3-methallyl or 2- or 3-butenyl; lower alkadienyl is, for example, 1-penta-2,4-dienyl; lower alkynyl is, for example, propargyl or 2-butynyl. In corresponding unsaturated radicals, the double bond is located especially in a position higher than the α-position with respect to the free valency. Substituents are especially the acyclic radicals mentioned above as substituents of R^o, preferably free or esterified carboxy, such as lower alkoxy-carbonyl, or di-lower alkylamino.

A cycloaliphatic radical R_1 having up to 29 carbon atoms is especially a substituted or, preferably, an unsubstituted mono-, bi- or poly-cyclic cycloalkyl, cycloalkenyl or cyclo-alkadienyl radical. Preferred are radicals having a maximum of 14, especially 12, ring carbon atoms and 3- to 8-, preferably 5- to 7- and especially 6-membered rings that may also carry one or more, for example two, aliphatic hydrocarbon radicals, for example those mentioned above, especially the lower alkyl radicals, or further cycloaliphatic radicals. Preferred substituents are the acyclic substituents mentioned above for R^o.

A cycloaliphatic-aliphatic radical R_1 having up to 29 carbon atoms is a radical in which an

acyclic radical, especially one having a maximum of 7, and preferably a maximum of 4, carbon atoms, such as especially methyl, ethyl and vinyl, carries one or more cycloaliphatic radicals having the meanings given above. There may be mentioned in particular cycloalkyl-lower alkyl radicals, and the analogues thereof that are unsaturated in the ring and/or chain but are non-aromatic and that carry the ring at the terminal carbon atom of the chain.

Heterocyclic radicals R_1 having up to 20 carbon atoms and up to 9 hetero atoms are especially monocyclic, but also bi- or poly-cyclic, aza-, thia-, oxa-, thiaza-, oxaza-, diaza-, triaza- or tetraza-cyclic radicals of aromatic character and corresponding partially saturated or, especially, completely saturated heterocyclic radicals of that kind, it being possible, where appropriate, for such radicals to carry further acyclic, carbocyclic or heterocyclic radicals and/or to be mono-, di- or poly-substituted by functional groups, preferably those mentioned above as substituents of aliphatic hydrocarbon radicals. The free valency of the heterocyclic radical R_1 must extend from one of its carbon atoms. They are especially unsubstituted or substituted monocyclic radicals having one nitrogen, oxygen or sulfur atom, such as 2-aziridinyl, and especially aromatic radicals of that kind, such as pyrryl, for example 2-pyrryl or 3-pyrryl, pyridyl, for example 2-, 3- or 4-pyridyl, or thienyl, for example 2- or 3-thienyl, or furyl, for example 2-furyl; analogous bicyclic radicals having one nitrogen, oxygen or sulfur atom are, for example, indolyl, such as 2- or 3-indolyl, quinolyl, such as 2- or 4-quinolyl, isoquinolyl, such as 3- or 5-isoquinolyl, benzofuranyl, such as 2-benzofuranyl, chromenyl, such as 3-chromenyl, or benzothienyl, such as 2- or 3-benzothienyl; preferred monocyclic and bicyclic radicals having a plurality of hetero atoms are, for example, imidazolyl, such as 2-imidazolyl, pyrimidinyl, such as 2- or 4-pyrimidinyl, oxazolyl, such as 2-oxazolyl, isoxazolyl, such as 3-isoxazolyl, or thiazolyl, such as 2-thiazolyl, and benzimidazolyl, such as 2-benzimidazolyl, benoxazolyl, such as 2-benzoxazolyl, of quinazolyl, such as 2-quinazolyl. Corresponding partially saturated or, especially, completely saturated analogous radicals also come into consideration, such as 2-tetrahydrofuryl, 2- or 3-pyrrolidyl, 2-, 3- or 4-piperidyl, and also 2- or 3-morpholinyl, 2- or 3-thiomorpholinyl, 2-piperazinyl and N,N'-bis-lower alkyl-2-piperazinyl radicals. These radicals may also carry one or more acyclic, carbocyclic or heterocyclic radicals, especially those mentioned above.

Heterocyclic-aliphatic radicals R_1 are especially aliphatic radicals having a maximum of 7, preferably a maximum of 4, carbon atoms, for example those mentioned above, that carry one, two or more heterocyclic radicals, for example those mentioned above, it also

being possible for the heterocyclic ring to be bonded to the aliphatic chain by one of its nitrogen atoms. A preferred heterocyclic-aliphatic radical R_1 is, for example, pyrid-3-yl-methyl.

A heteroaliphatic radical R_1 having up to 20 carbon atoms and up to 10 hetero atoms is an aliphatic radical that contains, in place of one, two or more carbon atoms, identical or different hetero atoms, such as especially oxygen, sulfur and nitrogen. An especially preferred form of a heteroaliphatic radical R_1 is an oxaalkyl radical, in which, in a preferably linear alkyl, one or more carbon atoms have been replaced by oxygen atoms that are preferably separated from one another by a plurality of (especially 2) carbon atoms, so that they form an optionally repeatedly recurring group $(O\text{-}CH_2\text{-}CH_2\text{-})_n$ wherein n = 1 to 7.

The following radicals R_1 are most preferred: C_1-C_2alkyl substituted by lower alkoxycarbonyl, such as especially methoxycarbonyl, by carboxy, by pyridyl, such as especially pyrid-3-yl, or by di-lower alkylamino, such as especially diethylamino; or lower alkanoyl, such as especially acetyl, benzoyl, pyridylcarbonyl, such as especially nicotinoyl or isonicotinoyl, or pyrrolylcarbonyl, such as especially 2-pyrroyl.

An aliphatic radical R_2 is, for example, one of the radicals that is mentioned above for an aliphatic hydrocarbon radical R_1, but that, in contrast to the aliphatic hydrocarbon radical R_1, may also be unsubstituted and/or may contain, in place of one or more than one carbon atom, also hetero atoms.

A carbocyclic or carbocyclic-aliphatic radical R_2 having up to 29 carbon atoms includes the meanings mentioned above for a cycloaliphatic or cycloaliphatic-aliphatic radical R_1 and in addition, however, aromatic and aromatic-aliphatic radicals. An aromatic radical (aryl radical) R_2 is especially a phenyl, but also a naphthyl, such as 1- or 2-naphthyl, a biphenylyl, such as especially 4-biphenylyl, or also an anthryl, fluorenyl or azulenyl, or an aromatic analogue thereof having one or more saturated rings. Preferred aromatic-aliphatic radicals are aryl-lower alkyl and aryl-lower alkenyl radicals, for example phenyl-lower alkyl or phenyl-lower alkenyl having a terminal phenyl radical, for example benzyl, phenethyl, 1-, 2- or 3-phenylpropyl, diphenylmethyl (benzhydryl), trityl and cinnamyl, or also 1- or 2-naphthylmethyl. Of the aryl radicals that carry acyclic radicals, such as lower alkyl, there are to be mentioned especially o-, m- and p-tolyl and xylyl radicals having methyl radicals situated in different positions.

A heterocyclic-aliphatic radical R_2 has one of the meanings mentioned above for a heterocyclic-aliphatic radical R_1.

An acyl radical R_2 having up to 30 carbon atoms is formyl or has one of the meanings mentioned above for an acyl radical R_1 having from 2 to 30 carbon atoms.

The following radicals R_2 are preferred: hydrogen, C_1-C_2alkyl that is unsubstituted or substituted by lower alkoxycarbonyl, such as especially methoxycarbonyl or tertiary butoxycarbonyl, or by carboxy; or lower alkanoyl, such as especially acetyl, 2-(tetrahydropyran-4-yl)-oxy-lower alkanoyl, such as especially 2-(tetrahydropyran-4-yl)-oxy-propionyl or 2-(tetrahydropyran-4-yl)-oxy-acetyl, lower alkoxycarbonyl, such as especially tertiary butoxycarbonyl, or benzoyl.

Lower alkoxy R_3 is preferably methoxy.

Depending on their nature, the compounds according to the invention may, provided they contain salt-forming groups, also be in the form of salts, especially pharmaceutically acceptable, i.e. physiologically tolerable, salts. For isolation or purification purposes it is also possible to use pharmaceutically unsuitable salts. Only pharmaceutically acceptable salts are used therapeutically and these are preferred.

Thus, compounds of formula I having free acid groups, such as a free sulfo, phosphoryl or carboxy group, may be in the form of a salt, preferably a physiologically tolerable salt, with a salt-forming basic component. There come into consideration especially metal or ammonium salts, such as alkali metal and alkaline earth metal salts, for example sodium, potassium, magnesium or calcium salts, or ammonium salts with ammonia or suitable organic amines, especially tertiary monoamines and heterocyclic bases, for example triethylamine, tri-(2-hydroxyethyl)-amine, N-ethylpiperidine or N,N'-dimethylpiperazine.

Compounds according to the invention of basic character may also be in the form of addition salts, especially in the form of acid addition salts with inorganic and organic acids, but also in the form of quaternary salts. Thus, for example, compounds of formula I that carry a basic group, such as an amino group, as a substituent may form acid addition salts with commonly used acids. Suitable acids are, for example, hydrohalic acids, for example hydrochloric and hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid or perchloric acid, and aliphatic, alicyclic, aromatic or heterocyclic carboxylic or sulfonic

acids, such as formic, acetic, propionic, succinic, glycolic, lactic, malic, tartaric, citric, fumaric, maleic, hydroxymaleic, oxalic, pyruvic, phenylacetic, benzoic, p-aminobenzoic, anthranilic, p-hydroxybenzoic, salicylic, p-aminosalicylic, embonic, methanesulfonic, ethanesulfonic, hydroxyethanesulfonic, ethylenedisulfonic, halobenzenesulfonic, toluenesulfonic, naphthalenesulfonic acids or sulfanilic acid, also methionine, tryptophan, lysine or arginine, or also ascorbic acid.

The staurosporin derivatives of formula I are capable of fully re-sensitising multidrug-resistant cells to the action of anti-tumour agents, such as cytostatics, as is demonstrated in the Examples section of this text in the case of resistant human KB-8511 cells. Such anti-tumour agents are, for example, doxorubicin, daunorubicin, vincristine, etoposide, taxol, mitomycin C, actinomycin D, mitoxantrone and, especially, vinblastine and adriamycin. The staurosporin derivatives of formula I and pharmaceutically acceptable salts of such derivatives having at least one salt-forming group can therefore be used in combination with one of those anti-tumour agents for the treatment of tumour diseases.

As mentioned above, the inhibitory action of the compounds of formula I on protein kinase C virtually no longer exists or, compared with the analogous compounds wherein R_1 is hydrogen, is greatly weakened. To determine the protein kinase C inhibitory action, pig brain protein kinase C is used, which is purified in accordance with the procedure described by T. Uchida and C.R. Filburn in J. Biol. Chem. 259, 12311-4 (1984). The protein kinase C inhibitory action of the compounds of formula I was formerly determined according to the methodology of D. Fabbro *et al.*, Arch. Biochem. Biophys. 239, 102-111 (1985). The pig brain protein kinase C used according to the methodology mentioned is a mixture of different subtypes (isotypes) of protein kinase C. For that reason, nowadays, pure, recombinant isotypes of protein kinase C are mostly used instead of pig brain protein kinase C.

Recombinant PKC isotypes are cloned, expressed and purified as follows:

The preparation of various proteins with the aid of baculoviruses and their cloning and isolation from Sf9 insect cells is carried out as described by M.D. Summers and G.E. Smith, "A manual method for baculovirus vectors and insect cell culture procedure", Texas Agricul. Exptl. Station Bull. (1987), 1555. The construction and isolation of recombinant viruses for the expression of PKC-α (bovine), PKC-β1 (human), PKC-β2 (human) and PKC-γ (human/bovine hybrid) in Sf9 cells is carried out as described by

Stabel *et al.*, [S. Stabel, M. Liyanage and D. Frith, "Expression of protein kinase C isozymes in insect cells and isolation of recombinant proteins", Meth. Neurosc. (1993)]. The preparation of the PKC isotypes in Sf9 cells is carried out as specified by Stabel *et al.*, (see above), and the purification of the enzymes is performed by the method described in the publication by McGlynn *et al.*, [E. McGlynn, J. Liebetanz, S. Reutener, J. Wood, N.B. Lydon, H. Hofstetter, M. Vanek, T. Meyer and D. Fabbro, "Expression and partial characterization of rat protein kinase C-δ and protein kinase C-ζ in insect cells using recombinant baculovirus", J. Cell. Biochem. 49, 239-250 (1992)]. For the generation of recombinant PKC-δ (rat), PKC-ε (rat), PKC-ζ (rat) and PKC-η (mouse) and the expression and purification thereof the procedure described by Liyanage *et al.*, ["Protein kinase C group B members PKC-δ, -ε, -ζ and PKC-λ: Comparison of properties of recombinant proteins in vitro and in vivo", Biochem. J. 283, 781-787 (1992)] and McGlynn *et al.*, (see above) is followed, with the addition that, for the expression of PKC-η, the transfer vector pAc360 is used [V. Luckow and M.D. Summers, "Trends in the development of baculovirus expression", Biotechnology 6, 47-55 (1988)].

Measurement of the activity of the recombinant PKC isotypes obtained by the above method is carried out in the absence of lipid and calcium (co-factors). Protamine sulfate, which is phosphorylated in the absence of co-factors, is used as a substrate for this. The activity of the enzymes reflects the transfer of ^{32}P from γ-[^{32}P]-ATP to protamine sulfate. Protamine sulfate is a mixture of polypeptides that each comprise four C-terminal arginine residues. Measurement of the phosphate incorporation is carried out under the following conditions: 100 µl of the reaction mixture contain in final concentrations 20 mmol TRIS-HCl pH 7.4, 10 mmol $Mg[NO_3]_2$, 0.5 mg/ml protamine sulfate, 10 µmol ATP (0.1 µCi γ-[^{32}P]-ATP; 10 Ci/mol; Amersham, Little Chalfont, United Kingdom), various concentrations of inhibitory substances and 0.5-2.5 U (Units; one unit is the enzyme quantity that transfers one nanomol of ^{32}P from the above-mentioned γ-[^{32}P]-ATP to Histon H1 [Sigma, type V-S] in one minute per milligram of protein) of the enzymes. The reaction is initiated by adding the enzymes and transferring to 32°C. The reaction time is 20 minutes. Thereafter, the reaction is stopped by dropping aliquots of 50 µl onto P81 chromatography paper (Whatman, Maidstone, United Kingdom). After removing unbound γ-[^{32}P]-ATP and nucleotide fractions by washing procedures as described by J.J. Witt and R. Roskoski, "Rapid protein kinase assay using phospho-cellulose-paper absorption", Anal. Biochem. 66, 253-258 (1975), the phosphorylation of the substrate is determined by scintillation measurement. In this test, the compounds of formula I generally do not inhibit the various isotypes of protein kinase C (PKC) until they are at a concentration

IC_{50} that is greater by a factor of from about 20 to over 1000 than the IC_{50} values that are found for analogous compounds wherein R_1 is hydrogen.

Preferred are compounds of formula I wherein R_1 is C_1-C_2alkyl that is substituted by lower alkoxycarbonyl, such as especially methoxycarbonyl, by carboxy, by pyridyl, such as especially pyrid-3-yl, or by di-lower alkylamino, such as especially diethylamino, or is lower alkanoyl, such as especially acetyl, benzoyl, pyridylcarbonyl, such as especially nicotinoyl or isonicotinoyl, or pyrrolylcarbonyl, such as especially 2-pyrroyl, R_2 is hydrogen, C_1-C_2alkyl that is unsubstituted or substituted by lower alkoxycarbonyl, such as especially methoxycarbonyl or tertiary butoxycarbonyl, or by carboxy, or is lower alkanoyl, such as especially acetyl, 2-(tetrahydropyran-4-yl-oxy)-lower alkanoyl, such as especially 2-(tetrahydropyran-4-yl-oxy)-propionyl or 2-(tetrahydropyran-4-yl-oxy)-acetyl, lower alkoxycarbonyl, such as especially tertiary butoxycarbonyl, or benzoyl, and R_3 is hydroxy, lower alkoxy or, preferably, hydrogen or oxo, and salts of such compounds having at least one salt-forming group, with the exception of the compound of formula I wherein R_1 is methoxycarbonylmethyl, R_2 is benzoyl and R_3 is hydrogen.

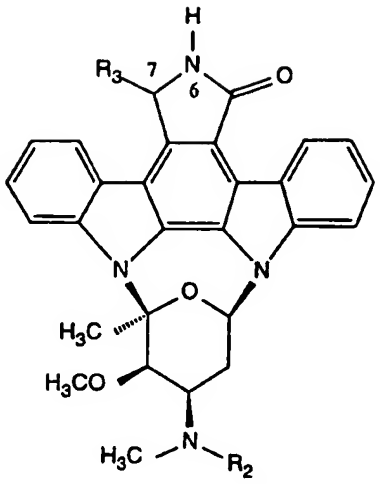
Especially preferred are the compounds of formula I described in the Examples and salts of such compounds having at least one salt-forming group.

Preferred in particular are compounds of formula I wherein R_2 is other than hydrogen, and salts of such compounds having at least one salt-forming group.

Most preferred are the compounds of formula I mentioned above and the subgroups of those compounds, given prominence as being preferred, wherein R_1 is other than lower alkanoyl, especially those compounds having virtually no significant inhibitory action on PKC, and salts of such compounds having at least one salt-forming group.

The compounds of formula I and salts of such compounds having at least one salt-forming group are prepared by processes known *per se*. The process according to the invention comprises

a) reacting a compound of formula II



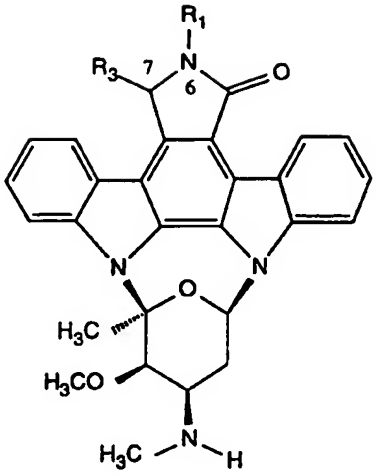
(II),

wherein the substituents are as defined above, any functional groups present in a compound of formula II being, if necessary, in protected form, or a salt of such a compound having at least one salt-forming group, with a compound of formula

R_1Y (III),

wherein R_1 is as defined above, any functional groups present therein being, if necessary, in protected form, and Y is a reactive activated hydroxy group or an additional single bond the other end of which replaces a hydrogen atom in the radical R_1, or with a salt of such a compound having at least one salt-forming group, and removing any protecting groups, or

b) reacting a compound of formula IV



(IV),

wherein the substituents are as defined above, any functional groups present therein being, if necessary, in protected form, or a salt of such a compound having at least one salt-forming group, with a compound of formula

R_2^aX (V),

wherein R_2^a has the meanings of R_2 mentioned above, with the exception of hydrogen, any functional groups present in the radical R_2^a being, if necessary, in protected form, and X is a leaving group or an additional single bond the other end of which replaces a hydrogen atom in the radical R_2^a, or with a salt of such a compound having at least one salt-forming group, and removing any protecting groups,

and, if desired, converting a resulting compound of formula I into a different compound of formula I and/or converting a compound of formula I obtained in free form into a salt thereof and/or converting a compound of formula I obtained in the form of a salt into its free form or into a different salt.

The way in which the above-mentioned process variants are carried out is explained in detail below:

General remarks:
The end products of formula I may contain substituents that can also be used as protecting groups in starting materials for the preparation of other end products of formula I. Within

the scope of this text, therefore, unless otherwise apparent from the context, the term "protecting group" denotes only a readily removable group that is not a component part of the particular desired end product of formula I.

Process a): Free functional groups that may be present in compounds of formulae II and III, which are preferably protected by readily removable protecting groups, are especially free amino or carboxy groups. It may also be advantageous to protect free hydroxy. Functional groups that are intended to participate in the desired reaction are not, of course, protected.

Protecting groups and the methods by which they are introduced and removed are described, for example, in "Protective Groups in Organic Chemistry", Plenum Press, London, New York 1973, and in "Methoden der organischen Chemie", Houben-Weyl, 4th edition, Vol. 15/1, Georg-Thieme-Verlag, Stuttgart 1974 and also in Theodora W. Greene, "Protective Groups in Organic Synthesis", John Wiley & Sons, New York 1981. It is characteristic of protecting groups that they can be removed easily, i.e. without undesirable secondary reactions taking place, for example by solvolysis, reduction, photolysis or also under physiological conditions.

A protected amino group may, for example, be in the form of a readily cleavable acylamino, arylmethylamino, etherified mercaptoamino, 2-acyl-lower alk-1-en-yl-amino, silylamino or stannylamino group or in the form of an azido group.

In a corresponding acylamino group, acyl is, for example, the acyl radical of an organic carboxylic acid having, for example, up to 18 carbon atoms, especially of an unsubstituted or substituted, for example halo- or aryl-substituted, alkanecarboxylic acid or an unsubstituted or substituted, for example halo-, lower alkoxy- or nitro-substituted, benzoic acid, or of a carbonic acid semiester. Such acyl groups are, for example, lower alkanoyl, such as formyl, acetyl or propionyl, halo-lower alkanoyl, such as 2-haloacetyl, especially 2-chloro-, 2-bromo-, 2-iodo-, 2,2,2-trifluoro- or 2,2,2-trichloro-acetyl, unsubstituted or substituted, for example halo-, lower alkoxy- or nitro-substituted, benzoyl, for example benzoyl, 4-chlorobenzoyl, 4-methoxybenzoyl or 4-nitrobenzoyl, or lower alkoxycarbonyl that is branched in the 1-position of the lower alkyl radical or suitably substituted in the 1- or 2-position, especially tert-lower alkoxycarbonyl, for example tert-butoxycarbonyl, arylmethoxycarbonyl having one or two aryl radicals which are preferably phenyl that is unsubstituted or is mono- or poly-substituted, for example, by lower alkyl, especially tert-

lower alkyl, such as tert-butyl, lower alkoxy, such as methoxy, hydroxy, halogen, for example chlorine, and/or by nitro, such as unsubstituted or substituted benzyloxycarbonyl, for example 4-nitrobenzyloxycarbonyl, or substituted diphenylmethoxycarbonyl, for example benzhydryloxycarbonyl or di-(4-methoxyphenyl)-methoxycarbonyl, aroyl-methoxycarbonyl wherein the aroyl group is preferably benzoyl that is unsubstituted or substituted, for example, by halogen, such as bromine, for example phenacyloxycarbonyl, 2-halo-lower alkoxycarbonyl, for example 2,2,2-trichloroethoxycarbonyl, 2-bromoethoxy-carbonyl or 2-iodoethoxycarbonyl, or 2-(trisubstituted silyl)-ethoxycarbonyl wherein each substituent independently is an aliphatic, araliphatic, cycloaliphatic or aromatic hydro-carbon radical having up to 15 carbon atoms that is unsubstituted or substituted, for example, by lower alkyl, lower alkoxy, aryl, halogen or by nitro, such as corresponding, unsubstituted or substituted, lower alkyl, phenyl-lower alkyl, cycloalkyl or phenyl, for example 2-tri-lower alkylsilylethoxycarbonyl, such as 2-trimethylsilylethoxycarbonyl or 2-(di-n-butyl-methyl-silyl)-ethoxycarbonyl, or 2-triarylsilylethoxycarbonyl, such as 2-tri-phenylsilylethoxycarbonyl.

Other acyl radicals that are suitable as amino-protecting groups are also corresponding radicals of organic phosphoric, phosphonic or phosphinic acids, such as di-lower alkyl-phosphoryl, for example dimethylphosphoryl, diethylphosphoryl, di-n-propylphosphoryl or diisopropylphosphoryl, dicycloalkylphosphoryl, for example dicyclohexylphosphoryl, unsubstituted or substituted diphenylphosphoryl, for example diphenylphosphoryl, unsub-stituted or substituted, for example nitro-substituted, di-(phenyl-lower alkyl)-phosphoryl, for example dibenzylphosphoryl or di-(4-nitrobenzyl)-phosphoryl, unsubstituted or substi-tuted phenyloxy-phenyl-phosphonyl, for example phenyloxyphenyl-phosphonyl, di-lower alkylphosphinyl, for example diethylphosphinyl, or unsubstituted or substituted diphenyl-phosphinyl, for example diphenylphosphinyl.

In an arylmethylamino group that is a mono-, di- or, especially, a tri-arylmethylamino group, the aryl radicals are, especially, unsubstituted or substituted phenyl radicals. Such groups are, for example, benzyl-, diphenylmethyl- and, especially, trityl-amino.

An etherified mercapto group in an amino group protected by such a radical is especially arylthio or aryl-lower alkylthio wherein aryl is especially phenyl that is unsubstituted or substituted, for example, by lower alkyl, such as methyl or tert.-butyl, lower alkoxy, such as methoxy, halogen, such as chlorine, and/or by nitro. A corresponding amino-protecting group is, for example, 4-nitrophenylthio.

In a 2-acyl-lower alk-1-en-1-yl radical that can be used as an amino-protecting group, acyl is, for example, the corresponding radical of a lower alkanecarboxylic acid, of a benzoic acid that is unsubstituted or substituted, for example, by lower alkyl, such as methyl or tert-butyl, lower alkoxy, such as methoxy, halogen, such as chlorine, and/or by nitro, or especially of a carbonic acid semiester, such as a carbonic acid lower alkyl semiester. Corresponding protecting groups are especially 1-lower alkanoyl-prop-1-en-2-yl, for example 1-acetyl-prop-1-en-2-yl, or 1-lower alkoxycarbonyl-prop-1-en-2-yl, for example 1-ethoxycarbonyl-prop-1-en-2-yl.

Preferred amino-protecting groups are acyl radicals of carbonic acid semiesters, especially tert-butoxycarbonyl, benzyloxycarbonyl that is unsubstituted or substituted, for example as indicated, for example 4-nitro-benzyloxycarbonyl, or diphenylmethoxycarbonyl, or 2-halo-lower alkoxycarbonyl, such as 2,2,2-trichloroethoxycarbonyl, also trityl or formyl.

Carboxy groups are usually protected in esterified form, such ester groupings being readily cleavable under mild conditions. Carboxy groups protected in that manner contain as esterifying groups especially lower alkyl groups that are branched in the 1-position or suitably substituted in the 1- or 2-position. Preferred carboxy groups in esterified form are *inter alia* tert-lower alkoxycarbonyl, for example tert-butoxycarbonyl, arylmethoxy-carbonyl having one or two aryl radicals which are phenyl radicals that are unsubstituted or mono- or poly-substituted, for example, by lower alkyl, such as tert-lower alkyl, for example tert-butyl, lower alkoxy, such as methoxy, hydroxy, halogen, for example chlorine, and/or by nitro, such as benzyloxycarbonyl that is unsubstituted or substituted, for example as mentioned above, for example 4-methoxybenzyloxycarbonyl or 4-nitro-benzyloxycarbonyl, or diphenylmethoxycarbonyl that is unsubstituted or substituted, for example as mentioned above, for example diphenylmethoxycarbonyl or di-(4-methoxy-phenyl)-methoxycarbonyl, 1-lower alkoxy-lower alkoxycarbonyl, such as methoxy-methoxycarbonyl, 1-methoxyethoxycarbonyl or 1-ethoxymethoxycarbonyl, 1-lower alkyl-thio-lower alkoxycarbonyl, such as 1-methylthiomethoxycarbonyl or 1-ethylthioethoxy-carbonyl, aroylmethoxycarbonyl wherein the aroyl group is benzoyl that is unsubstituted or substituted, for example by halogen, such as bromine, for example phenacyloxy-carbonyl, 2-halo-lower alkoxycarbonyl, for example 2,2,2-trichloroethoxycarbonyl, 2-bromoethoxycarbonyl or 2-iodoethoxycarbonyl, or 2-(trisubstituted silyl)ethoxycarbonyl wherein each substituent independently is an aliphatic, araliphatic, cycloaliphatic or aromatic hydrocarbon radical that is unsubstituted or substituted, for example, by lower

alkyl, lower alkoxy, aryl, halogen and/or by nitro, such as corresponding, unsubstituted or substituted, lower alkyl, phenyl-lower alkyl, cycloalkyl or phenyl, for example 2-tri-lower alkylsilylethoxycarbonyl, 2-trimethylsilylethoxycarbonyl or 2-(di-n-butyl-methyl-silyl)-ethoxycarbonyl, or 2-triarylsilylethoxycarbonyl, such as 2-triphenylsilylethoxycarbonyl.

The organic silyl and stannyl radicals mentioned above and hereinafter contain preferably lower alkyl, especially methyl, as substituents of the silicon or tin atoms. Corresponding silyl or stannyl groups are especially tri-lower alkylsilyl, especially trimethylsilyl, or dimethyl-tert-butyl-silyl, or correspondingly substituted stannyl, for example tri-n-butyl-stannyl.

Preferred protected carboxy groups are tert-lower alkoxycarbonyl, such as tert-butoxy-carbonyl, and especially benzyloxycarbonyl that is unsubstituted or substituted, for example, as mentioned above, such as 4-nitrobenzyloxycarbonyl, or diphenylmethoxy-carbonyl, especially 2-(trimethylsilyl)ethoxycarbonyl.

Hydroxy-protecting groups are, for example, acyl radicals, such as unsubstituted or substituted, for example halo-substituted, lower alkanoyl, such as 2,2-dichloroacetyl, or acyl radicals of carbonic acid semiesters, especially tert-butoxycarbonyl, unsubstituted or substituted benzyloxycarbonyl, for example 4-nitrobenzyloxycarbonyl, or diphenyl-methoxycarbonyl, or 2-halo-lower alkoxycarbonyl, such as 2,2,2-trichloroethoxycarbonyl, also trityl or formyl, or organic silyl or stannyl radicals, or readily removable etherifying groups, such as tert-lower alkyl, for example tert-butyl, 2-oxa- or 2-thia-aliphatic or -cycloaliphatic hydrocarbon radicals, especially 1-lower alkoxy-lower alkyl or 1-lower alkylthio-lower alkyl, for example methoxymethyl, 1-methoxy-ethyl, 1-ethoxy-ethyl, methylthiomethyl, 1-methylthioethyl or 1-ethylthioethyl, or 2-oxa- or 2-thia-cycloalkyl having 5 or 6 ring atoms, for example tetrahydrofuryl or 2-tetrahydropyranyl or corresponding thia analogues, and also unsubstituted or substituted 1-phenyl-lower alkyl, such as unsubstituted or substituted benzyl or diphenylmethyl, suitable substituents of the phenyl radicals being, for example, halogen, such as chlorine, lower alkoxy, such as methoxy, and/or nitro.

The removal of protecting groups that are not constituents of the desired end product of formula I, for example the carboxy-, amino-, hydroxy- or carbamoyl-protecting groups, is effected in a manner known *per se*, for example by means of solvolysis, especially hydrolysis, alcoholysis or acidolysis, or by means of reduction, especially hydrogenolysis or

chemical reduction, as appropriate stepwise or simultaneously, it being possible also to use enzymatic methods, for example acidolysis, such as treatment with trifluoroacetic acid or formic acid, or reduction, such as treatment with zinc and acetic acid, or with hydrogen and a hydrogenation catalyst, such as a palladium-on-carbon catalyst.

When several protected functional groups are present, the protecting groups are preferably so chosen that more than one such group can be removed simultaneously.

A protected amino group is freed in a manner known *per se* and, according to the nature of the protecting groups, in various ways, preferably by solvolysis or reduction. 2-Halo-lower alkoxycarbonylamino (where appropriate after conversion of a 2-bromo-lower alkoxycarbonylamino group into a 2-iodo-lower alkoxycarbonylamino group), aroyl-methoxycarbonylamino or 4-nitrobenzyloxycarbonylamino can be cleaved, for example, by treatment with a suitable chemical reducing agent, such as zinc in the presence of a suitable carboxylic acid, such as aqueous acetic acid. Aroylmethoxycarbonylamino can be cleaved also by treatment with a nucleophilic, preferably salt-forming, reagent, such as sodium thiophenolate, and 4-nitrobenzyloxycarbonylamino also by treatment with an alkali metal dithionite, for example sodium dithionite. Unsubstituted or substituted diphenylmethoxycarbonylamino, tert-lower alkoxycarbonylamino or 2-(tri-substituted silyl)-ethoxycarbonylamino, can be cleaved by treatment with a suitable acid, for example formic acid or trifluoroacetic acid, or with a saturated hydrochloric acid solution in ethyl acetate or dioxane; unsubstituted or substituted benzyloxycarbonylamino can be cleaved, for example, by means of hydrogenolysis, i.e. by treatment with hydrogen in the presence of a suitable hydrogenation catalyst, such as a palladium catalyst; unsubstituted or substituted triarylmethylamino or formylamino can be cleaved, for example, by treatment with an acid, such as a mineral acid, for example hydrochloric acid, or an organic acid, for example formic, acetic or trifluoroacetic acid, where appropriate in the presence of water; and an amino group protected by an organic silyl group can be freed, for example, by means of hydrolysis or alcoholysis. An amino group protected by 2-haloacetyl, for example 2-chloroacetyl, can be freed by treatment with thiourea in the presence of a base, or with a thiolate salt, such as an alkali metal thiolate, of thiourea, and subsequent solvolysis, such as alcoholysis or hydrolysis, of the resulting condensation product. An amino group protected by 2-substituted silylethoxycarbonyl can be converted into the free amino group also by treatment with a salt of hydrofluoric acid that yields fluoride anions.

Tert-lower alkoxycarbonyl, lower alkoxycarbonyl substituted in the 2-position by an

organic silyl group or in the 1-position by lower alkoxy or by lower alkylthio, or unsubstituted or substituted diphenylmethoxycarbonyl can be converted into free carboxy, for example, by treatment with a suitable acid, such as formic acid or trifluoroacetic acid, where appropriate with the addition of a nucleophilic compound, such as phenol or anisole. Unsubstituted or substituted benzyloxycarbonyl can be freed, for example, by means of hydrogenolysis, i.e. by treatment with hydrogen in the presence of a metal hydrogenation catalyst, such as a palladium catalyst. In addition, suitably substituted benzyloxycarbonyl, such as 4-nitrobenzyloxycarbonyl, can be converted into free carboxy also by chemical reduction, for example by treatment with an alkali metal dithionite, such as sodium dithionite, or with a reducing metal, for example zinc, or a reducing metal salt, such as a chromium(II) salt, for example chromium(II) chloride, customarily in the presence of a hydrogen-yielding agent that, together with the metal, is capable of producing nascent hydrogen, such as an acid, especially a suitable carboxylic acid, such as an unsubstituted or substituted, for example hydroxy-substituted, lower alkanecarboxylic acid, for example acetic acid, formic acid, glycolic acid, diphenylglycolic acid, lactic acid, mandelic acid, 4-chloromandelic acid or tartaric acid, or in the presence of an alcohol or thiol, water preferably being added. By treatment with a reducing metal or metal salt, as described above, 2-halo-lower alkoxycarbonyl (where appropriate after conversion of a 2-bromo-lower alkoxycarbonyl group into a corresponding 2-iodo-lower alkoxycarbonyl group) or aroylmethoxycarbonyl can also be converted into free carboxy. Aroylmethoxycarbonyl can be cleaved also by treatment with a nucleophilic, preferably salt-forming, reagent, such as sodium thiophenolate or sodium iodide. Substituted 2-silylethoxycarbonyl can also be converted into free carboxy by treatment with a salt of hydrofluoric acid that yields the fluoride anion, such as an alkali metal fluoride, for example sodium or potassium fluoride, in the presence of a macrocyclic polyether ("crown ether"), or with a fluoride of an organic quaternary base, such as tetra-lower alkylammonium fluoride or tri-lower alkyl-arylammonium fluoride, for example tetraethylammonium fluoride or tetrabutylammonium fluoride, in the presence of an aprotic, polar solvent, such as dimethyl sulfoxide or N,N-dimethylacetamide.

A hydroxy group protected by a suitable acyl group, an organic silyl group or by unsubstituted or substituted 1-phenyl-lower alkyl is freed analogously to a correspondingly protected amino group. Hydroxy protected by unsubstituted or substituted 1-phenyl-lower alkyl, for example benzyl, is freed preferably by catalytic hydrogenation, for example in the presence of a palladium-on-carbon catalyst. A hydroxy group protected by 2,2-dichloroacetyl is freed, for example, by basic hydrolysis, and a hydroxy group etherified by

tert-lower alkyl or by a 2-oxa- or 2-thia-aliphatic or -cycloaliphatic hydrocarbon radical is freed by acidolysis, for example by treatment with a mineral acid or a strong organic carboxylic acid, for example trifluoroacetic acid. Hydroxy etherified by an organic silyl radical, for example trimethylsilyl, can also be freed with a salt of hydrofluoric acid that yields fluoride anions, for example tetrabutylammonium fluoride.

If Y is a reactive activated hydroxy group, Y is bonded to a saturated carbon atom or a carbonyl carbon atom in the radical R_1 and is then especially a reactive esterified hydroxy group, i.e. one that is esterified by a strong inorganic acid, such as a hydrohalic acid (for example hydrochloric, hydrobromic or hydriodic acid), by an oxygen-containing mineral acid, such as phosphoric acid and, especially, sulfuric acid, or by a strong organic, such as aliphatic or aromatic, sulfonic acid (for example methane- and ethane- or benzene-, p-toluene-, p-nitrobenzene- and p-chlorobenzene-sulfonic acid), for example halogen, such as especially chlorine.

If Y is an additional single bond the other end of which replaces a hydrogen atom in the radicals R_1, R_1Y is, for example, an alkene, especially one in which the double bond has been additionally activated by a structural peculiarity, as in 2-methylpropene, or by substitution, such as especially in acrylonitrile. Also included in the definition of Y is a single bond the other end of which is not bonded directly to a carbon atom in the radical R_1 but is bonded to a hetero atom occurring as a substituent, such as oxygen (for example in a hydroxy group) or nitrogen (in an amino group) (replacing a hydrogen atom of that group). Especially preferred reagents of that kind contain the α-epoxide (oxirane) or α-imine (aziridine) grouping and serve as an advantageous source of radicals R^o having a 2-hydroxyalkyl grouping or 2-aminoalkyl grouping, respectively.

If R_1 is an acyl radical, the reagent R_1Y is a reactive carboxylic acid derivative. Y therein is, for example, a reactive esterified hydroxy group, such as especially halogen. Such reactive carboxylic acid derivatives of formula III are especially reactive activated esters or reactive anhydrides, or also reactive cyclic amides, it also being possible for the activation of the carboxylic acid of formula R_1-OH used as acylating agent to be performed *in situ* in the presence of the compound of formula II.

Activated esters of acids are especially esters that are unsaturated at the linking carbon atom of the esterifying radical, for example of the vinyl ester type, such as vinyl esters proper (obtainable, for example, by transesterification of a corresponding ester with vinyl

acetate; activated vinyl ester method), carbamoylvinyl esters (obtainable, for example, by treatment of the corresponding acid with an isoxazolium reagent; 1,2-oxazolium or Woodward method), or 1-lower alkoxyvinyl esters (obtainable, for example, by treatment of the corresponding acid with a lower alkoxyacetylene; ethoxyacetylene method), or esters of the amidino type, such as N,N'-disubstituted amidino esters (obtainable, for example, by treatment of the corresponding acid with a suitable N,N'-disubstituted carbodiimide, for example N,N'-dicyclohexylcarbodiimide; carbodiimide method), or N,N-disubstituted amidino esters (obtainable, for example, by treatment of the corresponding acid with an N,N-disubstituted cyanamide; cyanamide method), suitable aryl esters, especially phenyl esters suitably substituted by electron-attracting substituents (obtainable, for example, by treatment of the corresponding acid with a suitably substituted phenol, for example 4-nitrophenol, 4-methylsulfonylphenol, 2,4,5-trichlorophenol, 2,3,4,5,6-pentachlorophenol or 4-phenyldiazophenol, in the presence of a condensation agent, such as N,N'-dicyclohexylcarbodiimide; activated aryl esters method), cyanomethyl esters (obtainable, for example, by treatment of the corresponding acid with chloroacetonitrile in the presence of a base; cyanomethyl esters method), thioesters, especially unsubstituted or substituted, for example nitro-substituted, phenylthio esters (obtainable, for example, by treatment of the corresponding acid with unsubstituted or substituted, for example nitro-substituted, thiophenols, *inter alia* by the anhydride or carbodiimide method; activated thiol esters method), amino or amido esters (obtainable, for example, by treatment of the corresponding acid with an N-hydroxyamino or N-hydroxyamido compound, for example N-hydroxysuccinimide, N-hydroxypiperidine, N-hydroxyphthalimide or 1-hydroxybenzotriazole, for example by the anhydride or carbodiimide method; activated N-hydroxy esters method), or silyl esters (which are obtainable, for example, by treatment of the corresponding acid with a silylating agent, for example hexamethyldisilazane, and which readily react with hydroxy groups but not with amino groups).

Anhydrides of acids may be symmetric or preferably mixed anhydrides of those acids, for example anhydrides with inorganic acids, such as acid halides, especially acid chlorides (obtainable, for example, by treatment of the corresponding acid with thionyl chloride, phosphorus pentachloride or oxalyl chloride; acid chloride method), azides (obtainable, for example, from a corresponding acid ester *via* the corresponding hydrazide and treatment thereof with nitrous acid; azide method), anhydrides with carbonic acid semiderivatives, such as corresponding esters, for example carbonic acid lower alkyl semiesters (obtainable, for example, by treatment of the corresponding acid with haloformic, such as chloroformic, acid lower alkyl esters or with a 1-lower alkoxycarbonyl-2-lower alkoxy-

1,2-dihydroquinoline, for example 1-lower alkoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; mixed O-alkylcarbonic acid anhydrides method), or anhydrides with dihalogenated, especially dichlorinated, phosphoric acid (obtainable, for example, by treatment of the corresponding acid with phosphorus oxychloride; phosphorus oxychloride method), or anhydrides with organic acids, such as mixed anhydrides with organic carboxylic acids (obtainable, for example, by treatment of the corresponding acid with an unsubstituted or substituted lower alkane- or phenyl-lower alkane-carboxylic acid halide, for example phenylacetic acid chloride, pivalic acid chloride or trifluoroacetic acid chloride; mixed carboxylic acid anhydrides method) or with organic sulfonic acids (obtainable, for example, by treatment of a salt, such as an alkali metal salt, of the corresponding acid with a suitable organic sulfonic acid halide, such as a lower alkane- or aryl-, for example methane- or p-toluene-sulfonic acid chloride; mixed sulfonic acid anhydrides method) and symmetric anhydrides (obtainable, for example, by condensation of the corresponding acid in the presence of a carbodiimide or 1-diethylaminopropyne; symmetric anhydrides method).

Suitable cyclic amides are especially amides having five-membered diazacycles of aromatic character, such as amides with imidazoles, for example imidazole (obtainable, for example, by treatment of the corresponding acid with N,N'-carbonyldiimidazole; imidazole method), or pyrazoles, for example 3,5-dimethylpyrazole (obtainable, for example, *via* the acid hydrazide by treatment with acetylacetone; pyrazolide method).

As mentioned, derivatives of acids used as acylating agents can also be formed *in situ*. Thus, for example, N,N'-disubstituted amidino esters can be formed *in situ* by reacting a mixture of the starting material of formula II and the acid used as acylating agent in the presence of a suitable N,N-disubstituted carbodiimide, for example N,N'-dicyclohexylcarbodiimide. It is also possible to form amino or amido esters of the acids used as acylating agents in the presence of the starting material of formula II that is to be acylated, by reacting a mixture of the corresponding acid and amino starting materials in the presence of an N,N'-disubstituted carbodiimide, for example N,N'-dicyclohexylcarbodiimide, and of an N-hydroxyamine or N-hydroxyamide, for example N-hydroxysuccinimide, N-hydroxy-norbornane-2,3-dicarboximide or N-hydroxybenzotriazole, where appropriate in the presence of a suitable base, for example 4-dimethylaminopyridine or tetramethylguanidine.

In order to introduce a radical R_1 that is other than acyl, Process a) is preferably carried

out by first reacting the starting material of formula II in a suitable solvent, such as dimethylformamide or tetrahydrofuran, with a suitable base, such as sodium bis(trimethylsilyl)amide in tetrahydrofuran or sodium hydride, at a temperature of preferably from -20°C to +70°C, especially from 0°C to room temperature, and then adding the compound of formula III, for example in a suitable solvent, such as tetrahydrofuran.

In order to introduce an acyl radical R_1, Process a) is preferably carried out by reacting the starting material of formula II in a suitable solvent, such as methylene chloride, in the presence of a suitable base, such as triethylamine, with a reactive acid derivative of formula III, which may also be formed *in situ* from the corresponding acid, at a temperature of from 0°C to +150°C, for example under reflux. Alternatively, the starting material of formula II can first be reacted in a suitable solvent, such as absolute tetrahydrofuran, with a suitable base, such as sodium bis(trimethylsilyl)amide in tetrahydrofuran, at a temperature of from 0°C to room temperature, and then a reactive acid derivative of formula III can be added.

Process b):

The functional groups to be protected in the reactants of formulae IV and V and the protecting groups used for that purpose correspond to those mentioned in Process a). Functional groups that are intended to participate in the desired reaction, such as the group $-NH-CH_3$, are not, of course, protected. The introduction and removal of the protecting groups is also carried out analogously to the manner described in Process a). In the case of non-aromatic radicals R_2, the leaving group X in a compound of formula V corresponds to the reactive activated hydroxy group Y in the compound of formula III and the reagents of formula V are analogous to the reagents of formula III. In the case of aromatic radicals R_2, the leaving group X is, for example, a diazonium group.

In order to introduce a radical R_2^a that is other than acyl, Process b) is preferably carried out by reacting the starting material of formula IV in a suitable solvent, such as dimethylformamide or a halogenated hydrocarbon, such as chloroform, in the presence of a suitable base, such as N,N-diisopropylethylamine, at a suitable temperature, such as room temperature or elevated temperature up to about +150°C, with a compound of formula V, the reaction being carried out at elevated temperature, for example under pressure in a closed vessel, such as a bomb tube, especially when X is an additional single bond the other end of which replaces a hydrogen atom in the radical R_2^a, for example when the compound of formula V is an oxirane or acrylonitrile. The reaction with oxiranes is preferably carried

out in a lower alkanol, such as ethanol, as solvent.

In order to convert a compound of formula I obtained by Process a) or b) into a different compound of formula I, for example an ester grouping can be hydrolysed to carboxy or a carbonyl group can be reduced. The said hydrolysis is carried out, for example, in a manner known *per se* with dilute, for example 2-normal, sodium hydroxide solution in a lower alkanol, such as ethanol, at room temperature, and can also be seen as the removal of a protecting group. For the reduction of a carbonyl group, including a carbonyl group forming part of an amide or lactam group, reducing agents that come into consideration are, for example, complex metal hydrides, such as alkali metal aluminium hydrides and, especially, alkali metal borohydrides, for example lithium aluminium hydride, potassium borohydride, lithium borohydride and, especially, sodium borohydride, and derivatives thereof wherein one or more hydrogen atoms have been replaced by alkoxy radicals or by cyano, for example methoxysodium borohydride, tri-(tert-butoxy)lithium borohydride or di-(2-methoxyethoxy)-disodium lithium hydride or sodium cyanoborohydride, and also diborane.

Salt-forming groups in compounds of formula II to V and salts thereof are those mentioned above for the compounds of formula I.

The salt formation, which is to be carried out if desired, or the freeing of the fundamental forms from their salts is carried out in a conventional manner that is generally known *per se*. Thus, compounds carrying carboxy groups are converted into corresponding salts with bases, especially into alkali metal salts, by treatment with a corresponding base, especially a compound giving an alkaline reaction, such as hydroxide, carbonate or bicarbonate.

The salts can be converted into free carboxy compounds by acidifying, for example with inorganic acids, such as especially hydrohalic acids. End products giving a basic reaction, for example amines, can be converted into their salts with acids, for example by treatment with an acid suitable for salt formation, such as one of those mentioned above; conversely, by treating with agents that give a basic reaction, such as with inorganic hydroxides, carbonates and bicarbonates, or organic bases and ion-exchangers, such a basic fundamental form of an amine is freed.

Salts, such as the picrates, can also be used for the purification of the compounds obtained, by converting the free compounds into salts, separating these and recovering the free

compounds from the salts again.

In view of the close relationship between the compounds in free form and in the form of their salts, hereinbefore and hereinafter any reference to the free compounds is to be understood as including also the corresponding salts (including quaternary salts) where appropriate and expedient.

The starting materials corresponding to the formula IV wherein, however, R_1 is hydrogen are known or can be prepared by processes that are known *per se*. The starting material corresponding to the formula IV wherein R_1 and R_3 are each hydrogen, i.e. staurosporin, is commercially available and can be obtained by fermentation with the strain Streptomyces staurosporeus. That strain was deposited under number FERM P-3725 at the Fermentation Research Institute, Japan, in connection with Japanese Examined Patent Publication [Kokoku] No. 57-53076 which was published on 11.11.1982, see S. Omura *et al.*, J. Antibiot. 30, 275-281 (1977). Staurosporin derivatives corresponding to formula IV wherein R_3 is other than hydrogen are, for example, described by I. Takahashi *et al.*, J. Pharmacol. Exp. Ther. 255(3) (1990) 1218-1221 and in WO-A-8907-105-A (Applicant: Kyowa Hakko Kogyo KK, Japanese Priority No. 024571 of 4. 2. 1988). Compounds of formula II wherein R_3 is oxo are obtained, for example, from the corresponding compounds of formula II wherein R_3 is hydrogen by oxidation with chromium trioxide in pyridine. From the 7-oxo compounds so obtained the corresponding 7-hydroxy compounds wherein R_3 is hydroxy are obtained by reduction with sodium borohydride. Compounds corresponding to formula I wherein R_3 is hydroxy or oxo are also obtained as a by-product in the synthesis of compounds of formula I wherein R_3 is hydrogen. From the known staurosporin derivatives the starting materials of formulae II and IV that are still novel are obtained by appropriately carrying out reactions that are analogous to Process variants a) and b) described above.

The starting materials of formula V wherein R_2^a is 2-(tetrahydropyran-4-yl-oxy)-lower alkanoyl are obtained, for example, by reacting tetrahydropyran-4-ol with a corresponding chloro-lower alkanoic acid. In this procedure, tetrahydropyran-4-ol is first reacted in a suitable inert aprotic solvent, such as an acyclic or cyclic ether, such as dioxane, with a suitable base, such as sodium hydride. The suspension so obtained is added dropwise to a solution of a chloro-lower alkanoic acid in a suitable inert aprotic solvent, such as an acyclic or cyclic ether, such as dioxane. The reaction is carried out at from O°C to 150°C, preferably from 20°C to 100°C, for example at the reflux temperature of the solvent used.

The compounds of formula I carrying a 2-(tetrahydropyran-4-yl-oxy)-lower alkanoyl radical are many times, for example more than 10 times, more soluble in water and other solvents than are other N-acyl-staurosporin derivatives, such as N-benzoylstaurosporin.

Unless stated otherwise, all of the processes described above, including the processes for removing protecting groups and the additional process measures, are carried out in a manner known *per se*, for example in the presence or absence of preferably inert solvents and diluents, if necessary in the presence of condensation agents or catalysts, at reduced or elevated temperature, for example in a temperature range of from approximately -70°C to approximately +150°C, especially from approximately -20°C to approximately +100°C, mainly from approximately 0°C to approximately +70°C, preferably from approximately 0°C to approximately +50°C, mainly at room temperature, in a suitable vessel and, if necessary, under an inert gas atmosphere, for example a nitrogen atmosphere.

In those processes, taking into consideration all of the substituents in the molecule, if necessary, for example if readily hydrolysable radicals are present, especially mild reaction conditions are to be used, such as short reaction times, the use of mild acidic or basic agents in low concentration, stoichiometric quantity ratios, selection of suitable catalysts, solvents and temperature and/or pressure conditions.

The invention relates also to those forms of the process in which a compound obtainable as intermediate at any stage of the process is used as starting material and the remaining steps are carried out, or the process is discontinued at any stage or a starting material is formed under the reaction conditions or is used in the form of a reactive derivative or salt. The starting materials used are preferably those which result in accordance with the process in the compounds described above as being especially valuable.

The present invention relates also to novel starting materials and/or intermediates and to processes for the preparation thereof. The starting materials used and the reaction conditions chosen are preferably such that the compounds mentioned in this Application as being especially preferred are obtained.

The invention relates also to the use of the compounds of formula I and their pharmaceutically acceptable acid addition salts, preferably in the form of pharmaceutical compositions, for the therapeutic treatment of the human or animal body, especially in the case of

the diseases mentioned above. The invention relates also to a method of removing existing multidrug resistance and of preventing the development of multidrug resistance in a warm-blooded animal in need of such treatment, wherein an effective dose that removes the multi-drug resistance and avoids the development thereof of a compound of formula I, or of a pharmaceutically acceptable salt thereof, is administered enterally, for example orally, or parenterally, for example intraperitoneally or intravenously, to that warm-blooded animal. The dose of the active ingredient depends *inter alia* upon the nature of the disease, the species to be treated and its size, the organism's state of defence and the mode of administration. For example, a daily dose of from 10 mg to 1000 mg, mainly from 50 mg to 500 mg, preferably from 70 mg to 300 mg, for example 150 mg, of a compound of formula I will be administered, for example enterally, such as orally, or parenterally, such as intravenously or intraperitoneally, to a warm-blooded animal of approximately 70 kg body weight. This total daily dose may be divided into 2 or 3 doses per day.

The invention relates also to pharmaceutical compositions that comprise an effective amount, especially an amount effective for the prophylaxis or treatment of one of the diseases mentioned above, of the active ingredient together with pharmaceutically acceptable carriers that are suitable for topical, enteral, for example oral or rectal, or parenteral, for example intravenous or intraperitoneal, administration, and may be inorganic or organic and solid or liquid. For oral administration there are used especially tablets or gelatin capsules that comprise the active ingredient together with diluents, for example lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycerol, and/or lubricants, for example silica, talc, stearic acid or salts thereof, such as magnesium or calcium stearate, and/or polyethylene glycol. Tablets may also comprise binders, for example magnesium aluminium silicate, starches, such as corn, wheat or rice starch, gelatin, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone, and, if desired, disintegrators, for example starches, agar, alginic acid or a salt thereof, such as sodium alginate, and/or effervescent mixtures, or adsorbents, colourings, flavourings and sweeteners. It is also possible to use the pharmacologically active compounds of the present invention in the form of parenterally administrable compositions or infusion solutions. Such solutions are preferably isotonic aqueous solutions or suspensions, it being possible, for example in the case of lyophilised compositions that comprise the active ingredient on its own or together with a carrier, for example mannitol, for such solutions or suspensions to be made up prior to use. The pharmaceutical compositions may be sterilised and/or may comprise excipients, for example preservatives, stabilisers,

wetting agents and/or emulsifiers, solubilisers, salts for regulating the osmotic pressure and/or buffers. The pharmaceutical compositions in question, which may, if desired, comprise other pharmacologically active substances, such as antibiotics, are prepared in a manner known *per se*, for example by means of conventional mixing, granulating, confectioning, dissolving or lyophilising processes, and comprise approximately from 0.01 % to 90 %, and in the case of lyophilised compositions up to 100 %, especially from approximately 0.1 % to approximately 50 %, most especially from 1 % to 30 %, active ingredient(s), an active ingredient concentration below 1 % being especially suitable for compositions for topical administration.

The following Examples illustrate the invention without limiting it in any way. The R_f values are determined on silica gel thin-layer plates (produced by Merck, Darmstadt, Germany). The ratio of the eluants to one another in the eluant mixtures used is given in parts by volume (v/v), and temperatures are given in degrees Celsius. In the case of optical rotation, the concentration, c, of the substance in the solvent or solvent mixture is given as a percentage (weight/volume).

Within the scope of this text, the following nomenclature is used to specify the compounds of formula I: the nitrogen atom N-R_2 in the tetrahydropyran ring in formula I is designated "N". For example, N-BOC-staurosporin is a staurosporin derivative in which the radical R_2 is BOC. The nitrogen atom N-R_1, on the other hand, is designated "6", as will be apparent from the numbering given in formula I. For example, 6-methoxycarbonylmethyl-staurosporin is a staurosporin derivative in which the radical R_1 is methoxycarbonylmethyl.

Abbreviations:
BOC: tertiary butoxycarbonyl
DMF: dimethylformamide
HPLC: high-pressure liquid chromatography
THF: tetrahydrofuran

Example 1: 1.132 g (0.002 mol) of N-BOC-staurosporin are dissolved in 10 ml of dry dimethylformamide and, at room temperature under a nitrogen atmosphere, 2.2 ml of a 1-molar solution of sodium bis(trimethylsilyl)amide in tetrahydrofuran are added and stirring is carried out for one hour. There is then added to the dark-brown solution 0.4 ml

(0.0022 mol) of bromoacetic acid methyl ester, the reaction mixture being decolorised again. After 3 hours at room temperature, the reaction mixture is poured onto ice and extracted with ethyl acetate. The organic phase is washed with 0.1-normal hydrochloric acid and saturated sodium chloride solution, dried over magnesium sulfate and concentrated by evaporation. The crude product is purified by means of flash-chromatography (silica gel 60, methylene chloride:ethanol = 98:2), to yield N-BOC-6-methoxycarbonylmethyl-staurosporin; m.p. 193-195°C, R_f = 0.58 (methylene chloride:ethanol = 99:1).

Example 2: 450 mg (0.7 mmol) of N-BOC-6-methoxycarbonylmethyl-staurosporin are dissolved in 4 ml of ethyl acetate and treated with a saturated hydrochloric acid solution in ethyl acetate. After 3.5 hours at room temperature, the reaction mixture is concentrated by evaporation, and the residue is taken up in methylene chloride, washed with saturated sodium hydrogen carbonate solution and saturated sodium chloride solution, dried over sodium sulfate and concentrated by evaporation. The crude product is purified by means of flash-chromatography (silica gel 60, methylene chloride:ethanol = 95:5), to yield 6-methoxycarbonylmethyl-staurosporin; m.p. 200-210°C, R_f = 0.36 (methylene chloride/-ethanol = 95:5).

Example 3: 360 mg (0.67 mmol) of 6-methoxycarbonylmethyl-staurosporin (see Example 2) are dissolved in 8 ml of dimethylformamide, and 0.13 ml (0.76 mmol) of N,N-diisopropylethylamine and 0.074 ml (0.80 mmol) of bromoacetic acid methyl ester are added thereto. After 1 hour at room temperature, the reaction mixture is concentrated by evaporation under a high vacuum and the residue is purified by flash-chromatography (silica gel 60, methylene chloride:ethanol = 98:2), to yield N,6-di-(methoxycarbonylmethyl)-staurosporin; m.p. 135-138°C, R_f = 0.20 (methylene chloride:ethanol = 98:2).

Example 4: Starting from 160 mg (0.26 mmol) of N,6-di-(methoxycarbonylmethyl)-staurosporin (see Example 3), there is obtained by hydrolysis with 0.35 ml of 2-normal sodium hydroxide solution in 10 ml of ethanol, after acidification with 0.16 ml of glacial acetic acid, dilution with 5 ml of water and filtration of the precipitate, N,6-di-(carboxymethyl)-staurosporin; m.p. 235-240°C, R_f = 0.12 (methylene chloride:methanol:glacial acetic acid = 50:50:1).

Example 5: In a round-bottomed flask equipped with an argon balloon, 6 mg of sodium hydride (80 %) are added at 0°C to 20 mg of N-benzoyl-staurosporin in 2 ml of THF and the mixture is stirred for 15 minutes. 30 mg of diethylaminoethyl chloride hydrochloride

in 800 µl of THF are then added and the reaction mixture is stirred first for one day at room temperature and then for a day at 40°C. 5 ml of 1-normal HCl are then added thereto and extraction is carried out with methylene chloride. The organic phase is washed with water and dried with a silicone folded filter, and the solvent is removed. Final purification is carried out by preparative HPLC on silica gel (Lichrosorb Si 60, 5 µm; 8 x 250 mm; methylene chloride:isopropanol:triethylamine = 99.2:0.8:0.05; 4 ml/min.; 300 nm; retention time 6.1 min.; 8 runs) and yields, after removal of the solvent, colourless N-benzoyl-6-(2-diethylaminoethyl)-staurosporin; m.p. 231-234°C.

Example 6: 12.7 µl of triethylamine and 12.3 mg of pyrrole-2-carboxylic acid chloride (see Beilstein Vol. 12, Supplement II, page 492) are added to a solution of 10 mg of N-benzoyl-staurosporin in 0.6 ml of dry methylene chloride. The solution is stirred under reflux for 2 days. The reaction solution is partitioned between 20 ml of methylene chloride and 20 ml of saturated sodium carbonate solution, the organic phase is washed with a small amount of water and dried with a silicone folded filter and the solvent is removed *in vacuo*. After preparative HPLC on silica gel (Lichrosorb Si 60, 10 µm; 4.6 x 250 mm; methylene chloride:hexane = 90:10, water saturated; 2 ml/min.; 280 nm; retention time 9 min.; 5 runs) and removal of the solvent, almost colourless N-benzoyl-6-(2-pyrroyl)-staurosporin is obtained; m.p. 215-220°C.

Example 7: A solution of 40 mg of N-benzoyl-staurosporin, 75 mg of isonicotinic acid chloride hydrochloride and 100 µl of triethylamine in 5 ml of dry methylene chloride is stirred under reflux for 5 hours. The reaction solution is partitioned between 20 ml of methylene chloride and 20 ml of saturated sodium carbonate solution, the organic phase is washed with a small amount of water and dried with a silicone folded filter and the solvent is removed *in vacuo*. The crude mixture is subjected to preliminary purification over a small column of silica gel (LiChroprep Si 60, 15-25 µm; 7 ml column; methylene chloride:hexane:triethylamine = 75:25:0.1, 50 ml), all the eluting solvent being removed *in vacuo*. Final purification is carried out by preparative HPLC on silica gel (Lichrosorb Si 60, 5 µm; 8 x 250 mm; methylene chloride:hexane:triethylamine = 80:20:0.08; 5 ml/min.; 280 nm; retention time 9.2 min.; 6 runs) and, after removal of the solvent, yields almost colourless N-benzoyl-6-isonicotinoyl-staurosporin; m.p. 222-230°C.

Example 8: A solution of 50 mg of N-benzoyl-staurosporin, 100 mg of nicotinic acid chloride hydrochloride and 120 µl of triethylamine in 7 ml of dry methylene chloride is stirred under reflux for 5 hours. The reaction solution is partitioned between 20 ml of

methylene chloride and 20 ml of saturated sodium carbonate solution, the organic phase is washed with a small amount of water and dried with a silicone folded filter and the solvent is removed. The crude mixture is subjected to preliminary purification over a small column of silica gel (LiChroprep Si 60, 15-25 µm; 10 ml column; methylene chloride:-hexane:triethylamine = 80:20:0.1, 30 ml), all the eluting solvent being removed *in vacuo*. Final purification is carried out by preparative HPLC on silica gel (Lichrosorb Si 60, 5 µm; 8 x 250 mm; methylene chloride:hexane:triethylamine = 80:20:0.08; 5 ml/min.; 280 nm; retention time 10.3 min.; 9 runs) and, after removal of the solvent, yields almost colourless N-benzoyl-6-nicotinoyl-staurosporin; m.p. 219-226°C.

Example 9: 20 mg of N-benzoyl-7-oxo-staurosporin are dissolved in 2 ml of DMF. After the addition of 3 mg of sodium hydride (80 %), the mixture is stirred at room temperature for ten minutes. 4 mg of diethylaminoethyl chloride hydrochloride in 500 µl of DMF are then added and stirring is carried out at 60°C for 20 hours. 5 ml of 0.1-normal HCl are added to the reaction mixture and extraction is carried out with methylene chloride. The organic phase is dried with a silicone folded filter and the solvent is removed. Final purification is carried out by preparative HPLC on silica gel (Lichrosorb Si 60, 5 µm; 16 x 250 mm; methylene chloride:isopropanol = 99:1, water saturated; 8 ml/min.; 300 nm; 15.8 min.; 2 runs) and yields yellow N-benzoyl-6-(2-diethylaminoethyl)-7-oxo-staurosporin; m.p. >260°C.

The starting material is obtained as follows (see Chem. Abstracts 112(9):77240s and PCT Int. Appl. WO 8907105 A1):
In a 100 ml round-bottomed flask, an oxidising agent, preferably chromium trioxide-pyridine complex (4.6 g in 50 ml of methylene chloride; Fieser & Fieser, Reagents for Organic Synthesis, Vol. 1, Wiley, 1967, page 145) is added at 0°C to 1 g of N-benzoyl-staurosporin in 10 ml of methylene chloride. The reaction mixture is stirred overnight at 4°-25°C, diluted with 250 ml of methylene chloride and extracted with water. The organic phases are combined and dried with a silicone folded filter, and the solvent is removed. After chromatography on silica gel (LiChroprep Si 60, 15-25 µm; methylene chloride:-isopropanol = 95:5, water saturated; 400 ml Büchi medium-pressure column, 44 ml/min., retention time 24 min.), N-benzoyl-7-oxo-staurosporin is obtained; m.p. 215-220°C.

Example 10: 700 mg of picolyl chloride hydrochloride are partitioned between 20 ml of methylene chloride and 5 ml of saturated sodium carbonate solution. The aqueous phase is washed with a small amount of methylene chloride, and the combined organic phases

are dried with a silicone folded filter, and the solvent is removed *in vacuo*. 86 mg of N-benzoyl-7-oxo-staurosporin in 5 ml of methylene chloride and 7 mg of sodium hydride (80%) are added and the reaction is maintained under reflux for 6 hours. The reaction mixture is partitioned between ice-cold 4-normal HCl and methylene chloride, and the organic phase is washed in succession with ice-water, 5 ml of saturated sodium carbonate solution and then with a small amount of water and is dried with a silicone folded filter, and the solvent is removed. Final purification is carried out by preparative HPLC on silica gel (Lichrosorb Si 60, 5 µm; 8 x 250 mm; methylene chloride:hexane:triethylamine = 80:20:0.08; 5 ml/min.; 280 nm; 13.5 min.; 18 runs) and yields N-benzoyl-7-oxo-6-(3-pyridyl-methyl)-staurosporin; m.p. 198-205°.

Example 11: In a three-necked flask equipped with a septum, a thermometer and an argon balloon, 283 mg (0.5 mmol) of N-BOC-staurosporin are dissolved, with the exclusion of light, in 5 ml of absolute tetrahydrofuran, and 0.51 ml of a 1-molar solution of sodium bis-(trimethylsilyl)amide in tetrahydrofuran (Fluka) is added thereto by means of a syringe. After stirring at room temperature for 15 minutes, 48 µl (0.505 mmol) of acetic anhydride are added and the reaction mixture is stirred at room temperature for 1 hour. For working up, it is diluted with ethyl acetate, washed with 0.1-normal hydrochloric acid, dried over sodium sulfate and concentrated by evaporation. The residue is purified by means of flash-chromatography on silica gel 60 (ethyl acetate:petroleum ether = 3:7), to yield N-BOC-6-acetyl-staurosporin; m.p. 255-260°C, R_f = 0.27 (methylene chloride:ethanol = 98:2).

Example 12: 8 ml of trifluoroacetic acid are added at 0°C under nitrogen and with the exclusion of light to 230 mg (0.38 mmol) of N-BOC-6-acetyl-staurosporin (see Example 11). After 15 minutes at 0°C, the reaction mixture is concentrated by evaporation and the residue is partitioned between ethyl acetate and saturated sodium hydrogen carbonate solution. The organic phase is dried over sodium sulfate and concentrated by evaporation, and the residue is purified by flash-chromatography on silica gel 60 (methylene chloride:ethanol = 98:2), to yield 6-acetyl-staurosporin; m.p. 185-188°C, R_f = 0.17 (methylene chloride:ethanol = 98:2).

Example 13: 150 mg (0.29 mmol) of 6-acetyl-staurosporin (see Example 12) are dissolved, with the exclusion of light, in 8 ml of chloroform (filtered before use through basic Alox [aluminium oxide]), and 56 µl (0.32 mmol) of N,N-diisopropylethylamine and 32 µl (0.34 mmol) of acetic anhydride are added thereto and the reaction mixture is stirred at

room temperature for 3 hours. It is then diluted with methylene chloride, washed with saturated sodium hydrogen carbonate solution and saturated sodium chloride solution, dried over sodium sulfate and concentrated by evaporation. Purification by means of flash-chromatography on silica gel 60 (methylene chloride:ethanol = 98:2) yields N,6-diacetyl-staurosporin; m.p. 285-287°C, R_f = 0.19 (methylene chloride:ethanol = 98:2).

Example 14: Analogously to Example 11, starting from N-BOC-staurosporin and using benzoic anhydride instead of acetic anhydride, N-BOC-6-benzoyl-staurosporin is obtained; melting range 200-210°C, R_f = 0.44 (methylene chloride:ethanol 98:2).

Example 15: Analogously to Example 12, starting from N-BOC-6-benzoyl-staurosporin, 6-benzoyl-staurosporin is obtained; m.p. 215-220°C, R_f = 0.12 (methylene chloride:-ethanol = 98:2).

Example 16: Analogously to Example 13, starting from 6-benzoyl-staurosporin and using benzoic anhydride instead of acetic anhydride, N,6-dibenzoyl-staurosporin is obtained; m.p. 220-225°C; R_f = 0.18 (methylene chloride:ethanol = 98:2).

Example 17: 90 µl (0.5 mmol) of N,N-diisopropylethylamine and 80 µl (0.5 mmol) of bromoacetic acid tert-butyl ester and a catalytic amount of potassium iodide are added under nitrogen and with the exclusion of light to a solution of 250 mg (0.44 mmol) of 6-benzoyl-staurosporin in 8 ml of dimethylformamide and the reaction mixture is stirred at room temperature for 20 hours. It is then concentration by evaporation and the residue is partitioned between ethyl acetate and saturated sodium hydrogen carbonate solution. The organic phase is dried over sodium sulfate and concentrated by evaporation, and the residue is subjected to flash-chromatography (silica gel 60, methylene chloride:ethanol = 98:2). 6-benzoyl-N-(tert-butoxycarbonyl-methyl)-staurosporin is obtained; m.p. 149-152°C, R_f = 0.43 (methylene chloride:ethanol = 98:2).

Example 18: At 0° and with the exclusion of light, 200 mg (0.29 mmol) of 6-benzoyl-N-(tert-butoxycarbonyl-methyl)-staurosporin is dissolved in 10 ml of trifluoroacetic acid and the solution is stirred at 0° for 30 minutes. After a further 14 hours at room temperature, the reaction solution is concentrated by evaporation, excess trifluoroacetic acid is removed by twice adding toluene and concentrating by evaporation and the residue is triturated with diethyl ether/ethyl acetate (8:2). The product is filtered off and dried under a high vacuum. The 6-benzoyl-N-carboxymethyl-staurosporin trifluoroacetate so obtained

comprises 1 mol of water and melts at 200-205°; R_f = 0.69 (isopropanol:water:pyridine:-acetic acid = 60:20:15:5).

Example 19: Analogously to Example 17, there is obtained from 6-benzoyl-staurosporin with ethyl iodide after a reaction time of 72 hours 6-benzoyl-N-ethyl-staurosporin having a melting point of 175-178°; R_f = 0.10 (methylene chloride:ethanol = 98:2).

Example 20: 43 mg (0.285 mmol) of 1-hydroxybenzotriazole and 55 mg (0.285 mmol) of N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) are added at 0°C under argon to a solution of 39 mg (0.219 mmol) of O-(tetrahydropyran-4-yl)-D-lactic acid in 5 ml of absolute dimethylformamide and the clear, colourless solution so obtained is stirred at 0°C for 3 hours. 100 mg (0.175 mmol) of 6-benzoyl-staurosporin (Example 15) are then added and the yellow solution so obtained is stirred for 2 hours at 0°C and for 18 hours at room temperature. 15 ml of water are then added to the yellow solution and the beige suspension so obtained is subsequently stirred for 1 hour at room temperature. The resulting crystals are filtered off with suction and washed with water. The crude product is purified by column chromatography on 30 g of silica gel (type Si 60, Merck 9385; 0.040-0.063 mm) in methylene chloride/methanol (98:2; 5 ml fractions). Fractions 16-22 are combined and concentrated by evaporation at 30°C under a high vacuum. The beige residue is further purified by preparative thick-layer chromatography (2 plates of Macherey-Nagel Sil-G200, UV 254) in methylene chloride/ethanol (95:5). The zone containing the desired product is scraped off the plate, suspended in 100 ml of methylene chloride/methanol (9:1), stirred for 1/2 hour, filtered and concentrated by evaporation at 30°C under a high vacuum. The residue crystallises from 6 ml of ethyl acetate/n-pentane (1:5). 6-Benzoyl-N-[O-(tetrahydropyran-4-yl)-D-lactoyl]-staurosporin is obtained in the form of beige crystals having a melting point of 287-289°C; $[\alpha]_D^{20}$ = +157.1 +/- 1.8° (c = 0.555; dimethylformamide), R_f = 0.50 (methylene chloride:-ethyl alcohol = 95:5), R_f = 0.53 (acetone), R_f = 0.81 (methylene chloride:methanol = 9:1).

Example 21: By coupling analogously to Example 20 there is obtained from 6-methoxy-carbonylmethyl-staurosporin and N-protected glycine (N-BOC-glycine) 6-methoxy-carbonylmethyl-N-(N-BOC-glycyl)-staurosporin; m.p. 183-188°C, R_f = 0.29 (toluene:-ethyl acetate = 6:4), R_f = 0.4 (methylene chloride:ethanol = 96:4).

Example 22: From 6-methoxycarbonylmethyl-N-(N-BOC-glycyl)staurosporin (see Example 21) there is obtained by removal of the amino-protecting group 6-methoxy-

carbonylmethyl-N-glycyl-staurosporin hydrochloride; m.p. 275-280°C, R_f = 0.37 (methylene chloride:ethanol:conc. ammonia = 95:5:0.1).

Example 23: Human KB-31 (sensitive) and KB-8511 (drug-resistant, P-glycoprotein [Pgp] overexpressing) cells are incubated under a 5 % carbon dioxide atmosphere in MEM-Alpha-Medium, with the addition of ribonucleosides and deoxyribonucleosides and in the presence of 5 % foetal calf serum, 50 units/ml of the antibiotic penicillin and 50 µg/ml of the antibiotic streptomycin. The KB-8511 cells are kept as stock in the presence of 10 ng/ml of the antineoplastically active substance Colcemid (demecolcine). To determine the inhibition of the cell growth, batches of 1500 cells (without the addition of Colcemid) are sown in 96-well microtitre plates and incubated overnight under the conditions mentioned above. The test substance (A: the antineoplastically active substance vinblastine, B: the compound of formula I N,6-diacetyl-staurosporin) is added in serial dilutions on day 1. The plates are then incubated under the conditions mentioned above for 4 days. During that time, the control cells undergo several cell divisions. After incubation, the cells are fixed with 3.3 % (w/v) aqueous glutaraldehyde solution, washed with water and stained with 0.05 % (w/v) methylene blue solution. After washing, the dye is eluted with 3 % (w/v) aqueous hydrochloric acid. The optical density (OD) per well, which is directly proportional to the number of cells, is then measured with a photometer at 665 nm. The IC_{50} values are calculated by means of a computer system, using the formula

$$[OD_{665}(test)-OD_{665}(start)]/[OD_{665}(control)-OD_{665}(start)] \times 100$$

The IC_{50} values are defined as being those concentrations of active ingredient at which the number of cells per well at the end of the incubation period amounts to only 50 % of the number of cells in the control cultures.

test substance [concentration]	% growth of KB 8511 cells:
A [400 ng/ml]	0
A [200 ng/ml]	30
A [100 ng/ml]	76
A [50 ng/ml]	87
A [25 ng/ml]	87
A [12.5 ng/ml]	90
A [6.25 ng/ml]	89
A [3.13 ng/ml]	90
B [1 µmol]	88
B [1 µmol] + A [400 ng/ml]	0
B [1 µmol] + A [200 ng/ml]	0
B [1 µmol] + A [100 ng/ml]	0
B [1 µmol] + A [50 ng/ml]	0
B [1 µmol] + A [25 ng/ml]	15
B [1 µmol] + A [12.5 ng/ml]	34
B [1 µmol] + A [6.25 ng/ml]	58
B [1 µmol] + A [3.13 ng/ml]	79
B [0.1 µmol]	91
B [0.1 µmol] + A [400 ng/ml]	0
B [0.1 µmol] + A [200 ng/ml]	14
B [0.1 µmol] + A [100 ng/ml]	68
B [0.1 µmol] + A [50 ng/ml]	91
B [0.1 µmol] + A [25 ng/ml]	92
B [0.1 µmol] + A [12.5 ng/ml]	95
B [0.1 µmol] + A [6.25 ng/ml]	97
B [0.1 µmol] + A [3.13 ng/ml]	100

test substance A: vinblastine

test substance B: N,6-diacetyl-staurosporin

Example 24: The following results are obtained analogously to Example 23, using test substance C (= N,6-di-(methoxycarbonylmethyl)-staurosporin) instead of test substance B:

test substance [concentration]	% growth of KB 8511 cells:
A [400 ng/ml]	0
A [200 ng/ml]	30
A [100 ng/ml]	76
A [50 ng/ml]	87
A [25 ng/ml]	87
A [12.5 ng/ml]	90
A [6.25 ng/ml]	89
A [3.13 ng/ml]	90
C [1 µmol]	90
C [1 µmol] + A [400 ng/ml]	0
C [1 µmol] + A [200 ng/ml]	0
C [1 µmol] + A [100 ng/ml]	0
C [1 µmol] + A [50 ng/ml]	0
C [1 µmol] + A [25 ng/ml]	0
C [1 µmol] + A [12.5 ng/ml]	0
C [1 µmol] + A [6.25 ng/ml]	0
C [1 µmol] + A [3.13 ng/ml]	0
C [0.1 µmol]	86
C [0.1 µmol] + A [400 ng/ml]	0
C [0.1 µmol] + A [200 ng/ml]	0
C [0.1 µmol] + A [100 ng/ml]	0
C [0.1 µmol] + A [50 ng/ml]	16
C [0.1 µmol] + A [25 ng/ml]	50
C [0.1 µmol] + A [12.5 ng/ml]	70
C [0.1 µmol] + A [6.25 ng/ml]	83
C [0.1 µmol] + A [3.13 ng/ml]	89

test substance A: vinblastine
test substance C: N,6-di-(methoxycarbonylmethyl)-staurosporine

Example 25: The following results are obtained analogously to Example 23, using test substance D (= N-BOC-6-methoxycarbonylmethyl-staurosporin) instead of test substance B:

test substance [concentration]	% growth of KB 8511 cells:
A [400 ng/ml]	0
A [200 ng/ml]	31
A [100 ng/ml]	83
A [50 ng/ml]	93
A [25 ng/ml]	93
A [12.5 ng/ml]	96
A [6.25 ng/ml]	101
A [3.13 ng/ml]	99
D [1 µmol]	92
D [1 µmol] + A [400 ng/ml]	0
D [1 µmol] + A [200 ng/ml]	0
D [1 µmol] + A [100 ng/ml]	0
D [1 µmol] + A [50 ng/ml]	0
D [1 µmol] + A [25 ng/ml]	0
D [1 µmol] + A [12.5 ng/ml]	0
D [1 µmol] + A [6.25 ng/ml]	0
D [1 µmol] + A [3.13 ng/ml]	0
D [0.1 µmol]	96
D [0.1 µmol] + A [400 ng/ml]	0
D [0.1 µmol] + A [200 ng/ml]	0
D [0.1 µmol] + A [100 ng/ml]	0
D [0.1 µmol] + A [50 ng/ml]	0
D [0.1 µmol] + A [25 ng/ml]	2
D [0.1 µmol] + A [12.5 ng/ml]	22
D [0.1 µmol] + A [6.25 ng/ml]	57
D [0.1 µmol] + A [3.13 ng/ml]	80

test substance A: vinblastine

test substance D: N-BOC-6-methoxycarbonylmethyl-staurosporin

Example 26: The determination of the *in vivo* antitumour activity of test substance D (= N-BOC-6-methoxycarbonylmethyl-staurosporin) against the drug-sensitive parental

KB-31 and a multidrug-resistant variant of the KB-31, i.e. the multidrug-resistant variant KB-8511, tumours is carried out in female Balb/c nude mice (Bomholdgaard, Copenhagen, Denmark) bearing serially passaged (minimum of three consecutive transplantations) either the human parental drug-sensitive KB-31 tumours or the human KB-8511 tumours. The KB-8511 tumours overexpress Pgb, the product of the mdr-1 gene (S. Akiyma et al., "Isolation and genetic characterization of human KB cell lines resistant to multiple drug", Somatic Cell and Mol. Gen. 11, 117-126 [1985]). Tumour fragments of approximately 25 mg are transplanted into the left flank of each animal (n = 6 per group). Treatment is started when the tumours reach a mean tumour volume of 150-200 mm^3: 10 mg/kg i.v. of the test substance D are given once as a single dose 4 hours prior to a single application of 9.0 mg/kg i.v. of adriamycin at day 7 after tumour transplantation. Tumour growth is monitored twice weekly by measuring perpendicular diameters. Tumour volumes are determined as described by T. Meyer *et al.*, Int. J. Cancer 43, 851-856 (1989), and expressed as relative tumour size (i.e. the increase in tumour volume compared with the tumour volume at the start of the treatment). Maximal tumour regression, expressed in %, i.e. the decrease in tumour volume compared with the volume at the start of the treatment, was reached by adriamycin (1 x 9 mg/kg i.v.) in the case of the KB-31 tumour and amounted to 24 % on day 5 after treatment.

As is evident from the quotient [%] of the median tumour volumes in the treated versus the control groups, i.e. the T/C-values [%], in the following table, test substance D sensitizes the multi-drug resistant KB-8511 tumours to adriamycin and restores the activity of adriamycin against the KB-8511 tumours to an extent similar to the activity of adriamycin against the adriamycin sensitive KB-31 tumours. The smaller the T/C values [%], the more active the given dose.

tumour	compound	dose [i.v.]	T/C [%]
KB-31	placebo	1 x 10 ml/kg	100
KB-31	substance D	1 x 10 mg/kg	89
KB-31	adriamycin	1 x 9 mg/kg	13
KB-8511	placebo	2 x 10 ml/kg	100
KB-8511	substance D	1 x 10 mg/kg	85
KB-8511	adriamycin	1 x 9 mg/kg	89
KB-8511	substance D and adriamycin	1 x 10 mg/kg 1 x 9 mg/kg	14

Example 27: Tablets, each comprising 20 mg of active ingredient, for example one of the compounds of formula I described in the preceding Examples, are prepared in the usual manner with the following composition:

Composition	
active ingredient	20 mg
wheat starch	60 mg
lactose	50 mg
colloidal silicic acid	5 mg
talc	9 mg
magnesium stearate	1 mg
	145 mg

Preparation: The active ingredient is mixed with a portion of the wheat starch, with the lactose and the colloidal silicic acid, and the mixture is forced through a sieve. A further portion of the wheat starch is made into a paste with 5 times the amount of water on a water bath and the powder mixture is kneaded with that paste until a slightly plastic mass has been produced.

The plastic mass is pressed through a sieve of approximately 3 mm mesh size and dried, and the resulting dry granules are forced through a sieve once more. The remainder of the wheat starch, the talc and the magnesium stearate are then added and the mixture is compressed to form tablets each weighing 145 mg and having a breaking notch.

Example 28: Capsules, each comprising 25 mg of active ingredient, for example one of the compounds of formula I described in the preceding Examples, are prepared as follows:

Composition	
active ingredient	25.0 mg
gelucire 44/14	183.3 mg

(gelucire 44/14 is a mixture of esters of saturated C_8-C_{18}-fatty acids with glycerol and polyethylene glycol having a molecular weight of approximately 1500; produced by: Gatte-fossé, F-69800 Saint Priest, France).

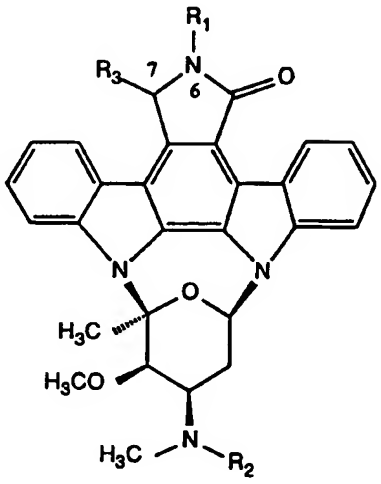
Preparation

A portion of the gelucire 44/14 is melted at a temperature of from 50°C to 100°C. The active ingredient is mixed with the liquid gelucire 44/14 in a heated mortar to form a paste. The remainder of the gelucire 44/14 is then also melted and is added to the paste. The mixture is stirred at 50°C until a solution is obtained. This is introduced into the capsules while warm and is cooled. The wax so obtained comprises 12 % by weight active ingredient.

The wax-like dispersion can also be processed in water by ultrasound treatment to form a milky liquid that can be administered orally.

What is claimed is:

1. A staurosporin derivative of formula I



(I),

wherein

R_1 is an acyl radical having from 2 to 30 carbon atoms, an aliphatic hydrocarbon radical having up to 29 carbon atoms that is substituted by acyclic substituents, a cycloaliphatic or cycloaliphatic-aliphatic radical each having up to 29 carbon atoms, or a heterocyclic, heterocyclic-aliphatic or heteroaliphatic radical each having up to 20 carbon atoms and up to 9 hetero atoms,

R_2 is hydrogen, an aliphatic, carbocyclic or carbocyclic-aliphatic radical each having up to 29 carbon atoms, a heterocyclic or heterocyclic-aliphatic radical each having up to 20 carbon atoms and up to 9 hetero atoms, or an acyl radical having up to 30 carbon atoms, and

R_3 is hydrogen, hydroxy, lower alkoxy or oxo,

or a salt of such a compound of formula I having at least one salt-forming group,

with the exception of the compound of formula I wherein R_1 is methoxycarbonylmethyl, R_2 is benzoyl and R_3 is hydrogen.

2. A compound of formula I according to claim 1, wherein

R_1 is C_1-C_2alkyl that is substituted by lower alkoxycarbonyl, by carboxy, by pyridyl, or by di-lower alkylamino, or is lower alkanoyl, benzoyl, pyridylcarbonyl or pyrrolylcarbonyl,

R_2 is hydrogen, C_1-C_2alkyl that is unsubstituted or substituted by lower alkoxycarbonyl or by carboxy, or is lower alkanoyl, 2-(tetrahydropyran-4-yl-oxy)-lower alkanoyl, lower alkoxycarbonyl or benzoyl, and

R_3 is hydroxy, lower alkoxy, hydrogen or oxo,

or a salt of such a compound having at least one salt-forming group,

with the exception of the compound of formula I wherein R_1 is methoxycarbonylmethyl, R_2 is benzoyl and R_3 is hydrogen.

3. A compound of formula I according to claim 1, wherein

R_1 is C_1-C_2alkyl that is substituted by methoxycarbonyl, by carboxy, by pyrid-3-yl or by diethylamino, or is acetyl, benzoyl, nicotinoyl, isonicotinoyl or 2-pyrroyl,

R_2 is hydrogen, C_1-C_2alkyl that is unsubstituted or substituted by methoxycarbonyl, tertiary butoxycarbonyl or by carboxy, or is acetyl, 2-(tetrahydropyran-4-yl-oxy)-propionyl or 2-(tetrahydropyran-4-yl-oxy)-acetyl, tertiary butoxycarbonyl or benzoyl, and

R_3 is hydrogen or oxo,

or a salt of such a compound having at least one salt-forming group,

with the exception of the compound of formula I wherein R_1 is methoxycarbonylmethyl, R_2 is benzoyl and R_3 is hydrogen.

4. A compound of formula I according to any one of claims 1 to 3, wherein R_1 is other than lower alkanoyl, or a salt of such a compound having at least one salt-forming group.

5. N-BOC-6-methoxycarbonylmethyl-staurosporin according to claim 1.

6. A compound of formula I according to claim 1, selected from

6-methoxycarbonylmethyl-staurosporin,

N,6-di-(methoxycarbonylmethyl)-staurosporin,

N,6-di-(carboxymethyl)-staurosporin,

N-benzoyl-6-(2-diethylaminoethyl)-staurosporin,

N-benzoyl-6-(2-pyrroyl)-staurosporin,

N-benzoyl-6-isonicotinoyl-staurosporin,

N-benzoyl-6-nicotinoyl-staurosporin,

N-benzoyl-6-(2-diethylaminoethyl)-7-oxo-staurosporin,

N-benzoyl-7-oxo-staurosporin,

N-benzoyl-7-oxo-6-(3-pyridyl-methyl)-staurosporin,

N-BOC-6-acetyl-staurosporin,
N-BOC-6-benzoyl-staurosporin,
6-benzoyl-staurosporin,
N,6-dibenzoyl-staurosporin,
6-benzoyl-N-(tert-butoxycarbonyl-methyl)-staurosporin,
6-benzoyl-N-carboxymethyl-staurosporin trifluoroacetate,
6-benzoyl-N-ethyl-staurosporin,
6-benzoyl-N-[O-(tetrahydropyran-4-yl)-(D)-lactoyl]-staurosporin and
N-[O-(tetrahydropyran-4-yl)-D-lactoyl]-6-benzoyl-staurosporin.

7. A compound of formula I according to claim 1, selected from
6-acetyl-staurosporin and
N,6-diacetyl-staurosporin.

8. A compound of formula I according to any one of claims 1 to 7 for use in a method for the therapeutic treatment of the human or animal body.

9. A pharmaceutical composition that comprises a compound of formula I according to any one of claims 1 to 7 together with pharmaceutical carriers.

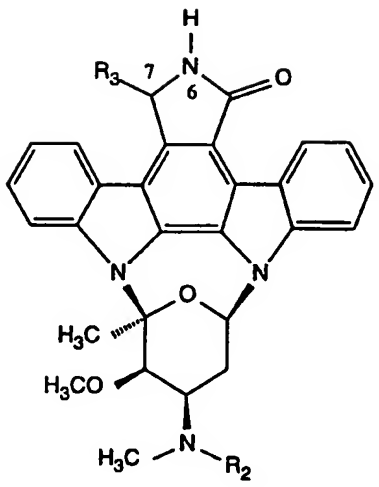
10. The use of a compound of formula I according to any one of claims 1 to 7 for avoiding or removing multi-drug resistance to anti-tumour agents.

11. The use of a compound of formula I according to any one of claims 1 to 7 for the preparation of pharmaceutical compositions intended for use in avoiding or removing multi-drug resistance to anti-tumour agents.

12. A method of avoiding or removing multi-drug resistance to anti-tumour agents in a warm-blooded animal in need of such treatment, wherein a dose of a compound of formula I according to any one of claims 1 to 7 that is effective for avoiding or removing multi-drug resistance to anti-tumour agents is administered to that warm-blooded animal.

13. A process for the preparation of a compound of formula I according to claim 1, or of a salt of such a compound having at least one salt-forming group, which comprises

a) reacting a compound of formula II



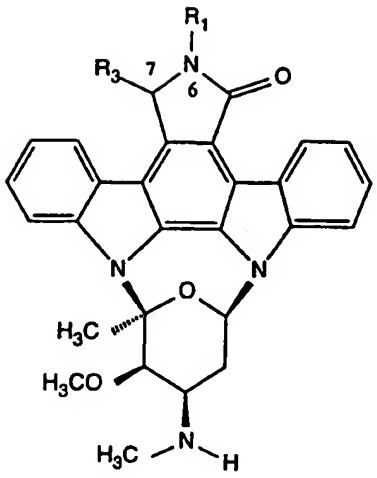
(II),

wherein the substituents are as defined above, any functional groups present in a compound of formula II being, if necessary, in protected form, or a salt of such a compound having at least one salt-forming group, with a compound of formula

$$R_1Y \qquad \text{(III)},$$

wherein R_1 is as defined above, any functional groups present therein being, if necessary, in protected form, and Y is a reactive activated hydroxy group or an additional single bond the other end of which replaces a hydrogen atom in the radical R_1, or with a salt of such a compound having at least one salt-forming group, and removing any protecting groups, or

b) reacting a compound of formula IV



(IV),

wherein the substituents are as defined above, any functional groups present therein being, if necessary, in protected form, or a salt of such a compound having at least one salt-forming group, with a compound of formula

R_2^aX (V),

wherein R_2^a has the meanings of R_2 mentioned above, with the exception of hydrogen, any functional groups present in the radical R_2^a being, if necessary, in protected form, and X is a leaving group or an additional single bond the other end of which replaces a hydrogen atom in the radical R_2^a, or with a salt of such a compound having at least one salt-forming group, and removing any protecting groups,

and, if desired, converting a resulting compound of formula I into a different compound of formula I and/or converting a compound of formula I obtained in free form into a salt thereof and/or converting a compound of formula I obtained in the form of a salt into its free form or into a different salt.

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]P 95/01910

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D498/22 C07H19/044 A61K31/55 A61K31/70
//(C07D498/22,311:00,273:00,209:00,209:00,209:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C07H A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 117, no. 23, 7 December 1992, Columbus, Ohio, US; abstract no. 234461n, R. YAMADA 'Preparation of staurosporine gamma-lactam derivatives and ammonium salts as blood platelet aggregation inhibitors' page 924 ; see abstract	1,9
X	& JP,A,4 145 085 (ASAHI)	1,9

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
3 August 1995	- 9. 08. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Voyiazoglou, D

Form PCT/ISA/210 (second sheet) (July 1992)

Inte[redacted]nal Application No
[redacted]EP 95/01910

C.(Continuation) DOCUMEN[redacted]ONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 116, no. 7, 17 February 1992, Columbus, Ohio, US; abstract no. 59419k, R. YAMADA ET AL 'Preparation of staurosporinecarboxylic acid derivatives as blood platelet aggragation inhibitors' page 869 ; see abstract	1,9
X	& JP,A,3 220 194 (ASAHI)	1,9
P,X	EP,A,0 630 898 (KYOWA) 28 December 1994 see claims 1,2	1,9
X	& WO,A,94 06799 31 March 1994	1,9
P,X	EP,A,0 643 966 (KYOWA) 22 March 1995 see claims 1,3,4	1,9,10
A	EP,A,0 383 919 (KYOWA) 29 August 1990 see page 1; claim 1	1,9
P,A	WO,A,95 00520 (CIBA-GEIGY) 5 January 1995 see page 4; claim 1	1,9,10

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB95/01910

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 12 is directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[illegible]nal Application No
[illegible]EP 95/01910

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
JP-A-4145085	19-05-92	NONE		
JP-A-3220194	27-09-91	NONE		
EP-A-0630898	28-12-94	CA-A-	2123895	03-03-94
		WO-A-	9406799	31-03-94
WO-A-9406799	31-03-94	CA-A-	2123895	03-03-94
		EP-A-	0630898	28-12-94
EP-A-0643966	22-03-95	WO-A-	9420106	15-09-94
EP-A-0383919	29-08-90	WO-A-	8907105	10-08-89
WO-A-9500520	05-01-95	AU-B-	7000094	17-01-95

Form PCT/ISA/210 (patent family annex) (July 1992)